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Thesis

THE RELATION OF INTERNAL SECRETIONS TO THE GROWTH OF BONE

by

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R.F.B.

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INTRODUCTION

The recognition of abnormalities of skeletal growth began far in the past, when giants and dwarfs were mentioned in legends and myths. The early Romans apparently recognized a difference between congenital dwarfism and the disproportionate dwarfism acquired later in life in their distinction between the _nanus_, or natural dwarf, and the _pumilo_, or unnatural dwarf. The physicians of olden times were well aware of the fact that castrates were taller than their fellow men. Beyond this, however, knowledge of the causes of gigantism and dwarfism did not progress until about the latter half of the nineteenth century, when wide-spread interest in the endocrine glands and their hormones led to many important discoveries. Retarded and accelerated skeletal growth were observed to accompany diseases or dysfunction of the endocrine glands, and these observations laid the foundation for systematic study of their effects on skeletal growth.

Studies were carried out by observing results of removal of the endocrine glands, of removal of the glands followed by transplantation of the glands or administration of their hormonal substances, and by transplantation of the glands and administration of hormones to normal animals (Silberberg and Silberberg, 1943).

Criteria for evaluating the influence of hormones on body growth usually have been changes in body weight, in over-all body length and in tail length of animals. In recent years, many investigators have studied the effects of the endocrine substances on the growth and

development of bone, and have been concerned with such sites of bone growth as the proximal end of the tibia, the costochondral junction of the ribs, and the vertebrae.

Since there is voluminous material on this subject, this paper will be limited to a discussion of the proximal end of the tibia, and will be confined mainly to the results of work on rats, although extensive work has been done on guinea pigs and mice, as well as on other animals.

NORMAL SEQUENCE OF EVENTS IN THE PROCESS OF BONE GROWTH

Detailed descriptions of the embryonic and later development of bone are to be found in all authoritative histology textbooks (Lambert, 1938; Jordan, 1940; Weatherford's edition of Bremer, 1944; and Maximow and Bloom, 1947), and give a much more complete picture of the processes involved than can be attempted here. However, it seems necessary to devote some space to the normal course of events in the growth of bone in order to be able later to make comparisons between the normal and abnormal conditions.

The development of bone begins rather late in embryonic life by one of two processes, or by a combination of bone. The flat bones of the face and the skull are formed by the process of intramembranous ossification, in which embryonic connective tissue is rather simply transformed into bone. The rest of the skeleton, however, is formed for the most part by the process of intracartilaginous or endochondral ossification, in which preformed embryonic cartilaginous plates are replaced by bone, together with intramembranous periosteal bone formation. Since we are primarily concerned here with the growth of the long bones of the body, particularly the tibia, the process of endochondral ossification will be presented in considerable detail.

Early Development of Bone

In the embryo, plates of hyaline cartilage are formed in much the same shape that the developing bone is to assume, and each

cartilaginous plate is surrounded by a layer of fibrous tissue, the perichondrium, which becomes the periosteum of the developing bone.

Bone formation begins at the primary center of ossification, about in the center of the cartilaginous plate. Here the cartilage cells begin to enlarge, and become arranged in rows extending out from the center of ossification, with thin transverse septa and wider longitudinal septa becoming prominent in the matrix. The cartilage cells then begin to degenerate, first becoming pyknotic and then karyolytic. While this is going on, the transverse and longitudinal septa of the cartilaginous matrix undergo calcification and then gradual absorption.

At approximately the same time that the primary center of ossification makes its appearance, osteoblasts develop along the inner portion of the perichondrium, and in the same manner that intramembranous bone is formed, lamellae of bone are laid down in a ring around the cartilage, forming the periosteal bone. From the inner cellular region of the periosteum, buds consisting of cells and fibers from the osteogenic layer, together with blood vessels, make their way toward the center of ossification, absorbing cartilaginous and bony substances as they go. By this time, absorption of the calcified cartilage has resulted in the formation of cavities which are penetrated by the periosteal buds, and the primary bone marrow cavity is formed. Other marrow cavities are formed until a large cavity fills the center of the diaphysis of the bone.

Cartilage cells continually enlarge and degenerate, the matrix becomes calcified, capillary loops from the marrow cavity invade the matrix followed by osteoblasts and osteoclasts, the calcified cartilage

is absorbed, bone is laid down in trabeculae and then the bony trabeculae are absorbed as growth proceeds and the bone becomes elongated. At the same time that the bone is growing in length, it is also growing in width.

While endochondral and perichondral ossification proceed, the epiphyseal cartilages at the ends of the cartilaginous plate continue to grow. After birth, however, ossification begins in the epiphyses. From the primary center of ossification in the diaphysis, osteogenic tissue makes its way into the cartilage, forming centers from which ossification proceeds in all directions, and results in the formation of spongy bone, together with some compact bone. As the centers of ossification in the diaphysis and the epiphyses expand and approach each other, a plate of unossified cartilage persists between them where new cartilage cells are continually formed. This insures growth of the bone until the animal reaches adulthood.

The Epiphyseal Cartilage

Since a great deal of the experimental work on the relation of internal secretions to the growth of bone has been concerned with changes in the proximal tibial epiphysis of the rat, a description of the normal conditions in this animal seems appropriate. Ingalls (1941) divides the proximal end of the tibia above the diaphysis into two regions roentgenographically and histologically -- the epiphysis and the metaphysis. The epiphysis consists of the epiphyseal center of ossification and the area of proliferating and growing cartilage cells. The latter, because it is uncalcified, is unaffected by silver impregnation staining techniques and does not cast a shadow on X-ray films (Fig. 1). Just below, the

Fig. 1. Structure of the proximal end of the tibia of the rat (lateral section) as seen when impregnated with silver. Silver supplants calcium in the calcified areas, and by reducing the silver phosphate thus formed to metallic silver and removing any unreacted calcium salts, black metallic silver remains at the calcified sites. (After T. H. Ingalls, 1941.)

metaphysis presents a contrast to the epiphysis. It consists of calcified cartilage and the primary spongiosa or bony encasement of the cartilage, and therefore becomes impregnated with silver and does cast a shadow on X-ray films. The metaphysis is the area of active endochondral bone formation, and merges with the secondary spongiosa of the diaphysis.

Identification of the various changes that occur in the proximal epiphyseal region of the rat's tibia has enabled investigators to detect the point of experimentally caused disturbances (Harris, 1933; Dodds and Cameron, 1934; Ingalls, 1941; Ray, Evans and Becks, 1941). The tibial epiphysis of the rat grows rapidly for some time after birth (Dodds and Cameron, 1934; Ingalls, 1941) and persists far into adulthood (Dawson, 1925). It lends itself well, therefore, to this type of study.

Five definite areas in the epiphyseal cartilage can be differentiated according to changes taking place. (Fig. 2).

1. Just below the epiphyseal bone can be seen a narrow <u>zone of remnants of the embryonic hyaline cartilage</u>, the cells of which multiply to form the mother cells which are to produce the cells in the cartilage columns.

2. Beneath the hyaline cartilage is an area where the cells from above repeatedly divide, forming columns containing a characteristic number of flattened cells, depending on the age of the animal. This <u>zone of proliferation</u> is the area which is responsible for the first increase in length of the bone.

3. The flattened cells then cease to multiply and grow rapidly, becoming round and full, and the intercellular cartilaginous matrix becomes reduced in amount. Here, in the <u>zone of cell growth</u>, the second and

Fig. 2. Schematic diagram of structure of proximal tibial epiphyseal cartilage of the rat. (From section stained with hematoxylin and eosin.)

greatest increase in length of the bone is accomplished. Although this zone is not very wide it can be recognized by the gradation in the size of the cells.

4. The zone of cell growth then merges with the <u>zone of calcification</u>. Here the mature cartilage cells begin to degenerate, first becoming pyknotic and then karyolytic, leaving large, empty lacunae, and calcium salts are deposited in the intervening matrix. Investigators differ as to the sites of deposition of the calcium salts, Dodds and Cameron (1934) indicating that the longitudinal septa but not the transverse septa become calcified. This seems to be the general opinion of several authors of histology texts (Lambert, 1938; Jordan, 1940; and Weatherford's edition of Bremer, 1944). Ingalls (1941) contradicts this and claims that calcification occurs in both. He has demonstrated the presence of calcium salts in both transverse and longitudinal septa by his silver impregnation technique, whereas Dodds and Cameron (1934) and other investigators have interpreted decalcified sections.

5. Beneath the zone of calcification can be distinguished the rather narrow <u>zone of erosion</u>. Capillary loops from the marrow cavity, followed closely by osteoblasts and osteoclasts, invade the empty lacunae, and the calcified cartilage is removed in some areas to be replaced by newly formed bone. The cartilage may also be incorporated into bony trabeculae laid down by the osteoblasts with formation of so-called osteoid. In this way the trabeculae of the diaphyseal bone are formed.

It must be borne in mind that all these changes are taking place simultaneously. There is an equilibrium established between proliferation of cartilage and removal of calcified cartilage and

deposition of bone, so that under normal conditions neither process precedes the other. In this way, the epiphysis is continually being pushed away from the diaphysis, and the newly formed diaphyseal trabeculae are constantly absorbed and formed again, revising and remodelling the growing bone.

Changes in the Epiphyseal Cartilage with Age

With advancing age, changes occur in the epiphyseal disc of the tibia. The disc becomes increasingly irregular, and is found by Becks, Simpson and Evans (1945) to decrease quite abruptly in width when the rate of body growth slows, from approximately 74 to 123 days of age, and then to decrease more slowly with increasing age. Ray, Evans and Becks (1941) find that the embryonic hyaline cartilage persists up to about 50 days of age, and then gradually begins to decrease until at about 150 days of age there are only scattered traces remaining. The columns of cartilage cells become shorter, due to decrease in the size and number of cells in the proliferating, growing and vesicular zones, and the amount of matrix increases. Activity in the zone of erosion and removal of calcified cartilage decreases, the bony trabeculae become coarser, more irregularly arranged and fewer in number, and are found to enclose large masses of cartilage including not only matrix but cells as well. At about 170 days of age, when growth has practically ceased (Becks, Simpson and Evans, 1945), bony trabeculae begin to form a continuous transverse ridge, thus sealing the epiphyseal cartilage off from the diaphysis. Even though the epiphysis becomes sealed off, however, the epiphyseal cartilage persists well into old age, and after 1100 days of age can still be seen as a peripheral ring (Dawson, 1925).

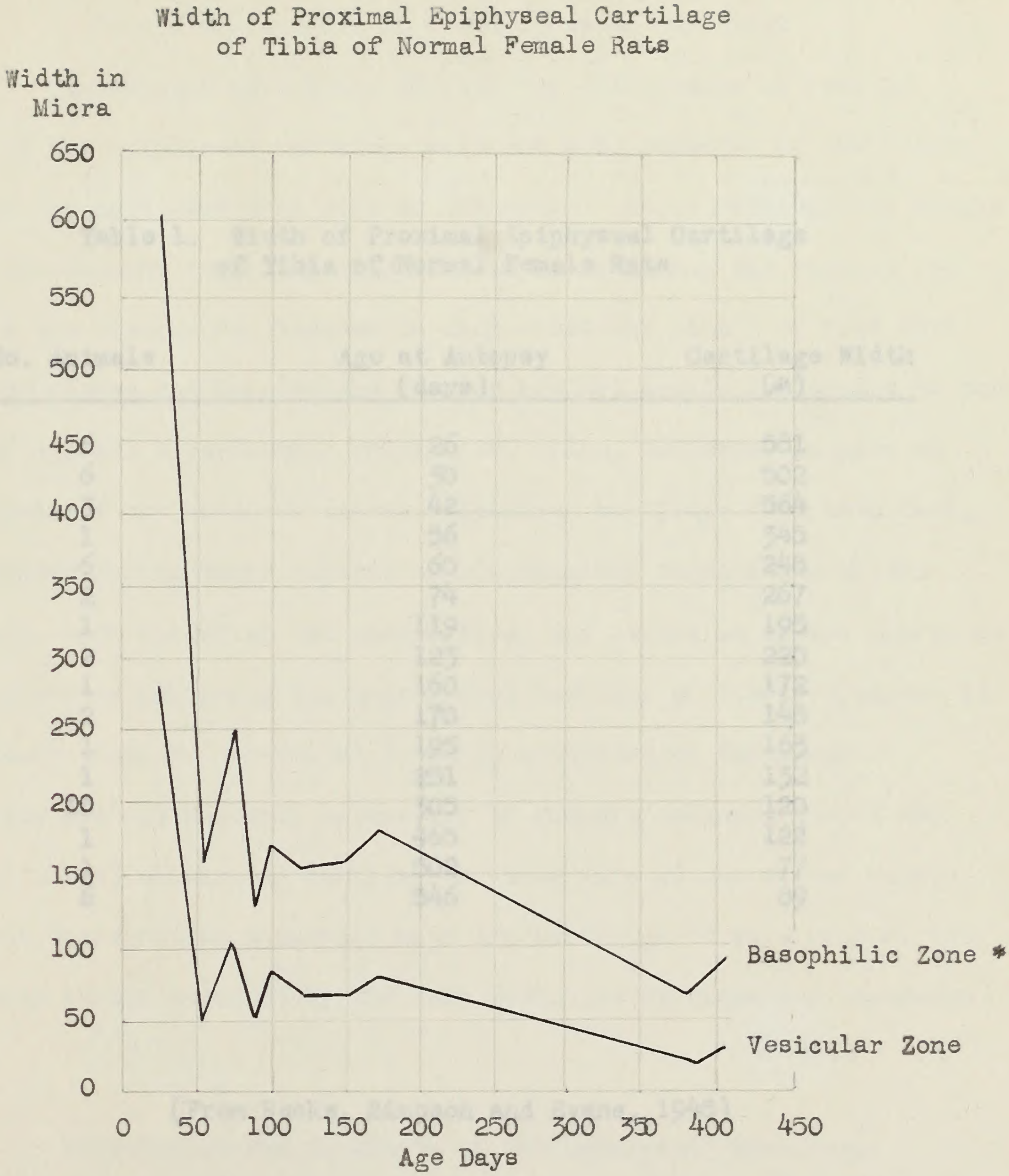

Fig. 3. Variation in Width of the Epiphyseal Cartilage with Age
(After Ray, Evans and Becks, 1941)

* The basophilic zone includes the zones of proliferation and cell growth described in this paper, and is therefore the uncalcified cartilage. The vesicular zone corresponds to the zone of calcification.

Table 1. Width of Proximal Epiphyseal Cartilage of Tibia of Normal Female Rats

No. Animals	Age at Autopsy (days)	Cartilage Width (μ)
6	26	581
6	30	502
3	42	564
1	56	345
6	65	248
2	74	267
1	119	195
2	123	220
1	160	172
2	170	145
1	195	163
1	251	132
15	305	120
1	465	122
1	502	77
6	546	89

(From Becks, Simpson and Evans, 1945)

Strain Differences in the Epiphyseal Cartilage

Comparisons of strains of rats for differences in rate of growth of the epiphyseal cartilage have not been reported in the literature, so far as I have been able to determine. It is difficult to arrive at any conclusions regarding this problem by examining the various reports in the literature, because in many cases the strain of rats used is not mentioned and because the results are not easily comparable on the basis of age and experimental procedures. Also, different methods of observation of the proximal tibial epiphyseal cartilage have been used, some workers basing their reports on histological examination of the cartilage, both calcified and uncalcified, and others on silver impregnation techniques involving the uncalcified portions of the cartilage. At the present time, an experiment is being conducted at the Boston University Biology Research Laboratory to compare measurements of the proximal tibial epiphyseal cartilage of male rats of the Wistar strain by silver impregnation technique with the cartilage of male rats of the Long-Evans strain measured by the same technique by Wyman and tum-Suden (1945).

Relation of Sex to Growth of the Epiphyseal Cartilage

The California group (Ray, Evans and Becks, 1941; Becks, Simpson and Evans, 1945), working on female rats of the Long-Evans strain, have observed changes in the epiphyseal cartilage at frequent intervals over long periods. Their results are best seen in Fig. 3 and Table 1. Measurements are based on histological sections, and include the entire cartilage. Wyman and tum Suden (1945) used the silver

impregnation technique to stain the epiphyseal cartilage of male and female rats of the Long-Evans hooded strain, and have found the growth curve over a period of 244 days to be essentially the same as that in the female rats studied by the Californians. Microscopic studies on male rats of various ages have been reported by many investigators, with no noticeable differences from those of female rats of the same age.

However, Saxton and Silberberg (1947), in studying the effects of diet on bone growth in rats of the Osborne Mendel (Yale) strain, have made observations on the sex differences in the skeletal development of normal rats. They have found that during the first months of life, skeletal development advances at a faster rate in the female than in the male. At 50 days of age, epiphyseal cartilage growth is more active in males than females, with more frequent mitosis in the proliferating cartilage. In females, hypertrophy and calcification of the cartilage are farther advanced, and the bony trabeculae in the metaphysis are more compact and mature. At 150 days of age, proliferation of cartilage cells decreases considerably, similar to the findings of Ray, Evans and Becks (1941) described previously, and is more pronounced in females than in males. Progress of ageing with advanced hyalinization of cells and matrix, and advanced bone formation, is more conspicuous in females than in males.

Whether or not similar differences between the sexes occur in other strains of rats may be questioned. No definite findings in this direction have been noted in reports of other workers. However, their investigations have not been primarily concerned with the problem of sex differences, as in Saxton's and Silberberg's work (1947), and it is quite

possible that a detailed study of skeletal development in males and females of other strains of rats may reveal comparable results.

The Importance of Diet in Maintaining Normal Growth

Saxton and Silberberg (1947), investigating the effects of diet on the growth of male and female rats of the Osborne Mendel (Yale) strain, find variations in the growth curve depending on sex (described previously) and diet. One group of rats received a basal diet adequate for normal growth with supplementary liver, milk, sugar or starch. Liver or milk in addition to the basal diet results in acceleration of growth and ageing of cartilage and bone to a greater degree than addition of sugar or starch. Another group of rats were underfed, receiving the basal diet in amount sufficient only to maintain constant body weight with a gain of about five grams in 50 days. In these rats, cartilage proliferation and especially hypertrophy are seen to be diminished, although growth still goes on at one year of age, showing that growth is slowed but not prematurely arrested. Regressive changes are not greatly delayed but proceed with less intensity. The over-all result is prolongation of the growth period. Comparison of the effects of adequate and inadequate diets may be seen in Table 2.

Similar results had been found by Day and Follis (1941) and Wyman and tum-Suden (1945) when diet was restricted in normal rats in their experiments on the effects of estrogens and adrenal insufficiency.

Table 2. The Effect of Diet on the Width of the Proximal Tibial Epiphyseal Cartilage of the Rat

Males

	Experimental Sample	Age in Days	Basal Starch	Diet Sugar	Supplemented Milk	With Liver	Underfed
	I	50	355	359	311	280	252
Width of	II	150	223	228	199	185	166
epiphyseal	III	250	165	163	171	134	171
cartilage	IV	350	150	153	191	131	177
(micra)	V	475	152	154	166	150	186
	VI	620	140	142	151	146	156
	VII	750	...	...	...	...	161

Females

	Experimental Sample	Age in Days	Basal Starch	Diet Sugar	Supplemented Milk	With Liver	Underfed
	I	50	334	348	338	322	263
Width of	II	150	197	202	184	144	176
epiphyseal	III	250	161	167	165	138	136
cartilage	IV	350	149	151	154	136	167
(micra)	V	475	156	162	181	171	153
	VI	620	142	144	146	151	149
	VII	750	...	...	...	...	157

(From Saxton and Silberberg, 1947)

HORMONES AND THEIR ACTIONS ON BONE GROWTH

The Influence of the Adrenal Glands

The first investigation of the effects of adrenalectomy on growth of the epiphyseal cartilage was carried out by Ingalls and Hayes in 1941. A group of eleven eight-week old albino rats of both sexes were bilaterally adrenalectomized, eight of which succumbed to adrenal insufficiency in ten days. The other three were given small subcutaneous injections of cortin for ten days, and then autopsied five to seven days after treatment had been stopped. In all animals the same changes were noted. Histological sections stained with hematoxylin and eosin and Masson's trichrome stain revealed that the proliferation of cartilage cells was inhibited, and that the columnar cartilage cells were stunted and irregular. The formation of bony trabeculae in the metaphyseal region was inhibited also, accompanied by decrease in the number of osteoblasts. At five to eight days after operation the primary spongiosa and ground substance of the degenerating cartilage cells had become quite flimsy, and the matrix was sparse and showed scanty, fragile calcification. Osteoid formation seemed to cease and existing bone began to atrophy. Roentgenograms of silver impregnated sections of the proximal epiphyseal cartilage, as well as the histological sections, showed marked deficiency of mineral salts, perhaps the most outstanding finding. The changes in the epiphyseal cartilage following adrenalectomy were found, therefore, to be very similar to, although more acute and widespread than, those following hypophysectomy.

In 1945, Wyman and tum-Suden bilaterally adrenalectomized male and female rats of the Long-Evans hooded strain at varying ages from 27 to 244 days, in an effort to amplify the suggestion of Ingalls and Hayes (1941) that adrenocortical atrophy and therefore insufficiency following hypophysectomy was a major factor in inhibition of epiphyseal cartilage growth. Since it had been shown by several investigators (Harris, 1933; Becks, Simpson, Li and Evans, 1944) that changes in the nutritional state of an animal causes changes in growth of the epiphyseal cartilage, and that severe loss of appetite occurs following adrenalectomy but can be restored by the administration of salt (Groat, 1941), Wyman and tum-Suden made observations on adrenalectomized rats with and without salt drinking water, and on rats placed on starvation and adequate diets. Micrometer ocular measurements of the epiphyseal cartilage were made at the proximal end of the split tibia which had been stained with silver nitrate (method of Evans, Simpson, Marx and Kibrick, 1943). Bilaterally adrenalectomized young rats on tap water showed proximal epiphyseal cartilages that were definitely narrower in width than those in normal rats of the same age. Mature adrenalectomized rats did not show any narrowing of the cartilage. In bilaterally adrenalectomized young rats given saline drinking water and in which appetite and food intake were maintained, the proximal epiphyseal cartilage was found to have increased considerably in width during about the first week and a half after operation. The widths of the epiphyseal cartilages of the rats placed on a starvation diet compared very favorably with those of adrenalectomized rats on tap water. (Fig. 4.)

These findings indicate, therefore, that widening of the proximal epiphyseal cartilage of the tibia occurs in young rats when an

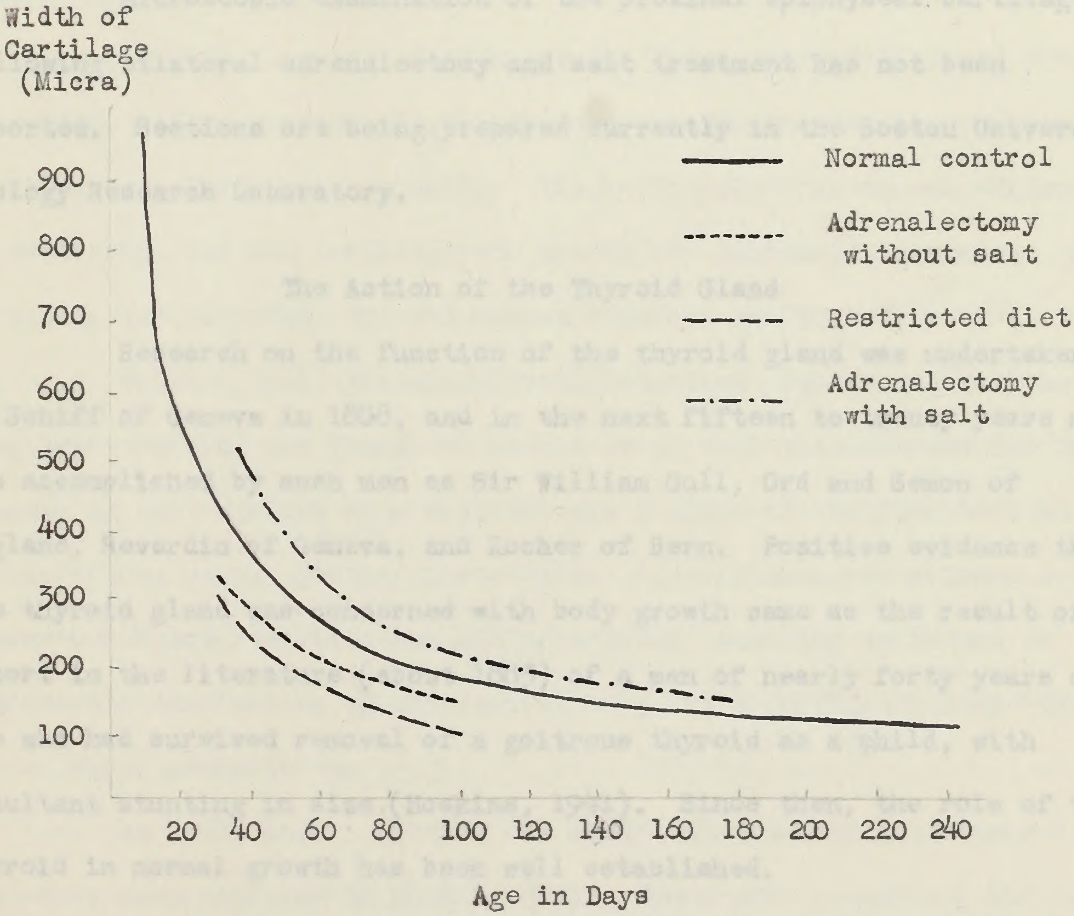

Fig. 4. The Effects of Adrenalectomy (With and Without Salt Treatment) and of Restricted Diet on the Epiphyseal Cartilage of the Rat (After Wyman and tum-Suden, 1945)

adequate food intake is maintained, in contrast to the decrease in cartilage width in adrenalectomized rats where inanition as the result of inadequate food intake occurs.

Microscopic examination of the proximal epiphyseal cartilage following bilateral adrenalectomy and salt treatment has not been reported. Sections are being prepared currently in the Boston University Biology Research Laboratory.

The Action of the Thyroid Gland

Research on the function of the thyroid gland was undertaken by Schiff of Geneva in 1858, and in the next fifteen to twenty years much was accomplished by such men as Sir William Gull, Ord and Semon of England, Reverdin of Geneva, and Kocher of Bern. Positive evidence that the thyroid gland was concerned with body growth came as the result of a report in the literature (about 1883) of a man of nearly forty years of age who had survived removal of a goitrous thyroid as a child, with resultant stunting in size.(Hoskins, 1941). Since then, the role of the thyroid in normal growth has been well established.

Until 1920, according to Hammett (1922), there had not been systematic investigation of the function of the thyroid as related to body growth as a whole. Since then, the problem has been approached from the point of view of growth in body length and weight change (Hammett, 1924, 1926a, 1926b; Salmon, 1936, 1938). Dott and Frazier in 1923 examined the epiphyseal cartilages of dogs and cats roentgenographically and histologically, and much work has been done since then on other animals.

Because of the intimate anatomical relationship of the thyroid

and parathyroid glands in the rat, removal of the thyroid without the parathyroids is a virtual impossibility. Therefore, in experiments where thyroidectomy has been performed, the parathyroid glands have always been removed also.

In 1941, Laqueur, Dingemanse and Freud thyroidectomized young male rats. After fourteen days they were sacrificed and the epiphyseal cartilage examined histologically. The cartilage cells showed evidence of dystrophy, and the cartilaginous matrix was increased in amount. Bone formation was retarded, but the marrow remained unaffected.

Salmon (1941) thyroparathyroidectomized newborn rats of the Long-Evans strain, and found two months later that the skeleton had progressed to and remained at a skeletal age (degree of ossification) of about fifteen days. Neither histological descriptions nor micrometer measurements were reported, but she apparently based her statement on microscopic examination of the ventral ribs and epiphyses of long bones, and on skull proportions.

In 1942, Becks, Kibrick and Evans thyroparathyroidectomized six young male rats (27 to 28 days old). These were sacrificed 468 to 522 days later, and the proximal tibial epiphyseal cartilages were examined histologically. Comparison of the epiphyseal cartilages of the thyroparathyroidectomized animals and their normal controls revealed striking differences. In the control animal of this age, the cartilage was shrunken and atrophied, and formed an irregular and sometimes discontinuous calcified line, sealed off from the diaphysis by a definite layer of bone. In the operated animal, however, the cartilage was of fairly even width and continuous, with a lesser degree of calcification

as indicated by the comparatively lighter stain with hematoxylin; the columns of cartilage cells were more regular and orderly and the cartilaginous matrix more abundant; and the diaphysis was not sealed off by a definite bony plate as in the controls, although discontinuous plates of bone and calcified cartilage frequently were present. Osteoblastic activity in both groups was practically nil. Micrometer measurements were not recorded.

The result, therefore, of thyroparathyroidectomy in young male rats is a general retardation of growth in long bones, with retarded maturation in the growth area as manifested by the retention of cartilaginous matrix in the proximal tibial epiphyseal cartilage, and slowing up of endochondral bone formation since the diaphysis was not completely sealed off from the epiphysis.

Becks, Ray, Simpson and Evans (1942) found essentially the same conditions in female rats thyroparathyroidectomized at 40 days of age. After 330 days, the cartilage cells were decreased in size, and the deposition of bone along the zone of erosion was diminished. They felt that the findings, although not as severe, resembled the picture of endochondral ossification following hypophysectomy.

Administration of thyroid to young and older rats with intact thyroids was carried out by Smith and McLean in 1938. The animals were fed diets containing between 0.4 and 0.75 per cent thyroid for four to twelve weeks, beginning at ages between one and a half and five and a half months of age. They were sacrificed between four and a half and eight months of age. Histological examination of the proximal tibial epiphyseal cartilage in eleven young rats treated during the active

growing period showed evidence of retarded endochondral bone formation. At this age (four and a half to five months) active endochondral bone formation in the normal rat was by proliferation and degeneration of cartilage cells, removal of calcified cartilage, and formation and reconstruction of bony trabeculae, with the presence of large numbers of osteoblasts and osteoclasts. In the treated animals, proliferation and degeneration of cartilage cells, and removal of calcified cartilage were greatly diminished, if not entirely so; the bony trabeculae had been absorbed and osteoblasts were practically absent. The cartilaginous plate was frequently perforated. Varying degrees of retarded growth were found, six showing complete retardation of growth, the other five showing a slight amount of growth. Of nine older treated rats killed at seven and eight months of age, retardation of growth was extreme or complete in seven animals, while two showed evidences of slight growth but continuous cartilaginous plates. Of nine control animals of this age, seven showed a moderate amount of growth, the other two presenting definite evidence of greatly retarded growth. Micrometer measurements of the epiphyseal cartilage were not made.

These results indicate, therefore, that toxic doses of thyroid cause a premature cessation or retardation of endochondral bone formation in the long bones.

Becks, Ray, Simpson and Evans (1942) gave injections of thyroxin over a period of 251 days to normal female rats and histologically found no definite stimulation of endochondral ossification. Micrometer measurements of the epiphyseal cartilage were not made.

These workers also gave injections of thyroxin after a post-

thyroidectomy period of 330 days to female rats operated at 35 to 45 days of age, and found that the microscopic appearance of the epiphyseal cartilage returned to normal. The dwarfing was repaired by symmetric stimulation of chondrogenesis and osteogenesis.

The Effects of the Parathyroid Hormone

Studies of the relationship of the parathyroid glands to the growth of bone apparently have been limited for the most part to the metabolism of calcium. However, some histological observations on the effects of hyper- and hypoparathyroidism on the epiphyseal junctions of the long bones have been made.

The parathyroid glands in the rat, while only two in number, may be found in a variety of positions, usually on the surface of the thyroid gland or embedded in it. Rarely is one of the pair found outside the thyroid (Burrows, 1938), and accessory parathyroids are seldom seen (Hoskins and Chandler, 1925). Therefore, parathyroidectomy, while presenting technical difficulties, may be performed with a fair degree of certainty.

Hammett (1922) parathyroidectomized male and female albino rats and compared his results with those of a series of thyroparathyroidectomized rats. His observations on body growth were based on body weight and tail and body length, and he found that growth was retarded to a somewhat lesser degree when the parathyroids alone were removed than when the thyroid and parathyroids together were removed.

In 1938, Salmon reported resumption of the normal growth curve did not result following administration of either thyroid or parathyroid hormones alone to thyroparathyroidectomized newborn rats of the

Long-Evans strain. Only when both substances were administered simultaneously were normal growth curves obtained.

According to Silberberg and Silberberg (1943), parathyroid hormone exerts an effect on the epiphyseal cartilage of mice similar to the effect exerted by thyroxin. However, it does not stimulate proliferation of cartilage cells, but promotes hypertrophy, calcification and disintegration of growing cartilage together with stimulation of formation of osteogenic tissue. This appears contradictory to the observations of Becks, Ray, Simpson and Evans (1942), who reported a return to normal in the microscopic appearance of the epiphyseal cartilage of thyroparathyroidectomized female rats following injection of thyroxin, with symmetric stimulation of chondrogenesis as well as osteogenesis. No mention was made of compensation for the parathyroid deficiency. A comparison of the effects of thyroparathyroidectomy and parathyroidectomy on the proximal tibial epiphysis of the rat, as well as the effects of administration of thyroxin and parathyroid hormone separately and in combination would be helpful in clarifying the situation.

Selye (1932) reported changes at the epiphyseal junction of the long bones of young rats following administration of parathormone. Microscopically he found the centers of ossification in treated animals consisting of a small area of bony tissue located in the center of the cartilaginous epiphysis. The epiphyseal cartilage was broad and irregular, and deposition of calcium was lacking in the ground substance. The bone in the center of the epiphysis consisted almost wholly of soft osteoid tissue, which appeared to account for the fact that bending invariably occurred in the region of the epiphyseal junction of the

bones of these rats.

In 1938, Burrows parathyroidectomized young growing rats and then administered parathyroid extract in varying dosages, and found retardation of growth, the amount of retardation depending upon the daily dosage. The first visible effect in the long bones was a decrease in the bony trabeculae, followed by an increase of the lamellae. Microscopically, there was resorption of bone. The osteoblasts were decreased in number and became changed to fibrous tissue. The osteoclasts increased in number. The result was the disease known as osteitis fibrosa. Osteoclastic activity followed the other processes and was therefore not the cause of decalcification, but rather the result of decalcification. When the injections were continued, these processes were reversed. The osteoclasts disappeared, osteoblasts reappeared in greater numbers, fibrous tissue diminished, and the bone began to show hypercalcification, resulting in the condition known as marble bone. If the injections were continued still longer, there was a tendency for the bones to return to a somewhat normal state, the osteoblasts and the size and number of trabeculae becoming moderately decreased. Burrows felt that the osteitis fibrosa condition was probably initiated by the parathyroid hormone itself, or by a substance set up by it in the blood, and that the marble bone condition which followed it was probably due to the development of an antibody-like substance in the blood causing hypercalcification as it acted to check decalcification. Then, another substance was probably formed in the blood stream to check hypercalcification, with the result that the bones of the animal tended to return to a normal condition. However, it was felt that since spontaneous hyperparathyroidism does not

initiate such a series of balances and counterbalances, the parathyroid extracts used contained other substances than the pure parathyroid hormone.

The Role of the Sex Hormones

In spite of the tremendous amount of work reported on the sex hormones each year, a comparatively small amount of information is available on the relation of sex hormones to the growth of bone. Several workers have been interested in the effects on the growth curve (Deanesly and Parks, 1941; Hooker and Pfeiffer, 1943), and the Silberbergs (1939a, 1939b, 1941 and 1946) have carried on quite extensive investigation of the effects of gonadectomy and treatment with the sex hormones on the proximal epiphyseal cartilage of the tibiae of guinea pigs and mice.

Studies of the effects of castration on growth in the rat have been limited largely to resultant changes in body weight and over-all body length (Rubinstein, Kurland and Goodwin, 1939; Rubinstein and Solomon, 1941a, 1941b). Turner, Lachmann and Hellbaum (1941), by means of X-ray examination, found no difference in the rate of growth of the long bones of young castrated and normal male rats as determined by differences in density, length of bones, and degree of epiphyseal union. Pomerat and Coe (1941) measured the long bones of young castrated male albino rats and found a decrease in the length of the long bones after a period of nearly one year. Tang (1941) measured the length of the tibiae and hip bones in gonadectomized albino male and female rats. He found a slower rate of growth in castrated males than in spayed females, the castrated males showing shorter lengths than their controls, and the

females showing greater lengths than their controls.

To my knowledge, the specific effects of gonadectomy in male and female rats on the growth of the proximal tibial epiphyseal cartilage are not reported. However, in 1939, the Silberbergs gonadectomized immature male and female guinea pigs and observed the effects on the cartilage and bone. They found both proliferative and regressive changes. Both hyperplasia and hypertrophy of cartilage cells were stimulated, as was calcification of the hypertrophic cartilage. Although cartilage proliferation was activated in both sexes, it was more intense in the male where hyperplastic growth was more accentuated, as compared with the females where hypertrophy was more evident. Calcification and ossification of cartilage appeared balanced in the females, but ossification was disturbed in the males with a tendency to fibrosis of the bone marrow. Again in 1940, the Silberbergs ovariectomized immature guinea pigs and examined the epiphyseal cartilage microscopically. During the first several months following operation, the width of the uncalcified cartilage became increased, but calcification and ossification of the cartilage were relatively retarded. Later, about eight or nine months postoperatively, the width of the cartilage decreased, although it was still relatively wider than that of the controls. Calcification and erosion of cartilage, and bone formation were intensified, although still somewhat decreased as compared with the normal controls.

The results of gonadectomy, therefore, would appear to be the maintenance of a youthful appearing epiphyseal cartilage, the degree of influence on growth of the bone in length depending upon the postoperative

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The results of gonadectomy, therefore, would appear to be the maintenance of a youthful appearing epiphyseal cartilage, the degree of influence on growth of the bone in length depending upon the postoperative

period and sex of the animal. However, the results of the experiments discussed when considered comparatively are quite confusing, and since the available material on this subject is so limited in amount, further investigation of the effects of gonadectomy on male and female rats, including comparison of body weight, body length and microscopic findings, as well as micrometer measurement of the proximal tibial epiphyseal cartilage, is needed.

The effects of the administration of estrogens on the epiphyseal cartilage were reported by Simpson, Kibrick, Becks and Evans in 1941. Three groups of young female rats were treated with subcutaneous pellets of crystalline alpha-estradiol dipropionate in varying amounts, and the proximal tibial epiphyseal cartilage measured (Table 3) and examined microscopically. In seven days, one implant of a 10 mg. estrin pellet resulted in marked narrowing of the epiphyseal cartilage. Osteoblastic activity, while still visible, was somewhat diminished as compared with the normal controls. The appearance of the cartilage was very much like that seen in starved animals. Since the animals showed evidences of marked weight loss and gross observation revealed marked anorexia, it was felt that inanition was a factor here, although the food intake had not been measured. When the animals received one implant of estrin pellets per week for three weeks, recovery of activity with resumption of the normal histological picture in the epiphyseal disc occurred. By the second or third week, food consumption in these animals compared favorably with that of the control animals, and it was felt that the decreased cartilage width (see Table 3) was due either to the effect of estrogen treatment alone or the residual effect

Table 3. The Effects of Varying Doses of Crystalline Estrin Implants on the Epiphyseal Cartilage of the Tibia of Young Female Rats

Group	Age at Autopsy (days)	Weight at Autopsy (gm.)	Width of Epiphyseal Cartilage (micra)
Normal control	75-76	189	257
10 mg. estradiol dipropionate s.c., 1 implant on 71st day	78	152	125
10 mg. estradiol dipropionate s.c., 3 weekly implants from 54th day	75	166	168
Normal control	96	225	176
10 mg. estradiol dipropionate s.c., 6 weekly implants from 54th day	96	194	120

(From Simpson, Kibrick, Becks and Evans, 1941)

of the initial reduction in food consumption. Hyperossification with marked osteoblastic activity was striking, thick anastomosing trabeculae obliterating the upper one-fifth of the marrow space which normally occupies the shaft of the bone. In animals receiving one implant per week for six weeks, the width of the epiphyseal cartilage was narrower than that of the normal controls, but the difference was not nearly so great as in the animals treated for three weeks. This shrinkage was felt to be most likely due to the effect of the excess estrogen or an endocrine imbalance rather than due to reduction in food consumption, although the latter factor could not be completely ruled out because of the absence of complete data. The bony trabeculae showed a greater degree of coalescence near the cartilaginous disc, while those farther away showed signs of resorption and reorganization into thicker and more widely spaced trabeculae. The results of estrogen treatment in this experiment, then, consisted of an involution of the proximal epiphyseal cartilage of the tibia of young adult female rats when treatment was initiated, with recovery of the growth capacity of the cartilage followed by hyperossification as treatment was continued, the extent of hyperossification being correlated with the length of treatment.

The Silberbergs (1941a) administered estrogen in oil subcutaneously to young male and female rats, beginning treatment at two weeks of age. They found that a smaller number of large doses over a short period of time were more effective than an equal amount given in smaller doses over a longer period of time. At the beginning of the experiment, 200 rat units were given. This was increased to 500 rat units after one month of treatment, with injection periods ranging from two to seventeen

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months. The treated animals at first showed only a very slow gain in weight, much less than their normal controls, and then no gain in weight for the duration of the experiment. Food consumption was not reported. They were much smaller and their bones much shorter than their normal controls. After four months of treatment with a total dose of 7300 rat units, the proximal tibial epiphyseal cartilage was markedly narrowed. Proliferation of cartilage cells was practically nil, and all cells throughout the cartilage were smaller than normal. In one cell row there were six or seven columnar cartilage cells as compared to nine or ten in the normal animal, and three hypertrophic cartilage cells as compared with four in the controls. The gradual transition of columnar into hypertrophic cartilage cells was inhibited, and the cartilaginous matrix was greatly increased in amount, sclerosed and hyalinized, or strongly calcified. The occurrence of osseous plugs traversing the epiphyseal disc from the metaphysis toward the epiphysis was not uncommon. In the normal rat, beginning formation of these plugs did not occur until the age of nine or ten months. After nine months of treatment with a total dose of 20,000 rat units, the epiphyseal cartilage was greatly narrowed, so much so that cell counts could not be made in many cases. In these cases, the disc was made up entirely of remnants of hyalinized cartilage and had become ossified over wide areas. In the cases where a cell count could be made, three or four columnar and two or three hypertrophic cartilage cells were counted as compared with eight columnar and three or four hypertrophic cells in normal animals of the same age. Preserved cartilage cells had undergone increased retrogressive changes, the matrix showed advanced sclerosis, and many hyalinized cartilage cells were

bone. The [illegible] in the [illegible] rat [illegible]

epiphyseal line than in the normal controls, and there were [illegible]

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[illegible] which [illegible] not uncommon. In the

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[illegible] under [illegible] regressive changes. The [illegible]

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directly converted into osteocytes. After sixteen to eighteen months of treatment with a total of 36,000 to 40,000 rat units, cell counts were impossible because of the extensive retrogressive changes and ossification. Epiphyseo-diaphyseal union had occurred, with absorption of much of the excessive bone present earlier. Although the cartilage was greatly narrowed in normal animals of the same age, five columnar and two hypertrophic cartilage cells could still be counted in a single column of cells. The results here are essentially the same as those found by Simpson, Kibrick, Becks and Evans (1941). When treatment was continued for a considerably long period of time the excessive bone formed in the earlier stages was absorbed. The end result is, therefore, a premature ageing of cartilage and bone formation with resultant stunting in size.

Day and Follis (1941) gave young rats large doses of estrogen for periods of 17 to 26 days. The proximal tibial epiphyseal cartilage was found to be thinner and the number of trabeculae in the metaphysis was increased, the amount of osseous tissue being greater in the females than in the males. Adult estrogen-treated rats showed similar changes in the proximal tibia, although not as extensive as in the younger animals.

Investigation of treatment of normal rats with testosterone propionate has been confined mainly to effects on body weight and overall body length, as in the case of castration. Some workers (Rubinstein, Kurland and Goodwin, 1939; Rubinstein and Solomon, 1941a) obtained a depressing or inhibitory effect on body growth when large amounts of the hormone were administered. However, when the hormone was given in

smaller doses, a stimulating effect on body growth resulted (Rubinstein, 1940; Rubinstein and Solomon, 1941b). Turner, Lachmann and Hellbaum (1940), again by X-ray examination, found no significant differences in the rate of skeletal development of rats treated with testosterone propionate, both normal and castrated, as compared with their normal controls.

Silberberg and Silberberg (1941b) gave testosterone propionate to immature female mice for periods ranging from two weeks to nineteen months. Histologically, the proximal tibial epiphyseal cartilage showed inhibition of proliferation and growth of cartilage cells in columns, intensification of degeneration of cartilage cells, and sclerosis, hyalinization and calcification of the cartilaginous matrix. During the early stages of administration of the hormone, absorption of bone was inhibited, but this effect was reversed as treatment continued and excess bone was removed followed by premature perforation of the epiphyseal disc. These effects did not continue indefinitely, but after a certain stage had been reached they came to a standstill, and later the epiphyseal cartilage resembled that seen in the normal animals. The age changes did not, as a rule, exceed those of the normal animal. High single doses of testosterone propionate were more effective than small single doses over a longer period of time in producing these effects. Later work (1946) by these investigators on mice revealed similar results in the action of testosterone propionate on the epiphyseal cartilage.

The Anterior Pituitary Hormones

The anterior pituitary gland has received more attention regarding the problem of bone growth than any other endocrine organ. The California groups have been the most active in studying the effects of anterior pituitary on the proximal tibial epiphyseal cartilage in the rat, although others have worked on the rat, guinea pig and mouse.

In 1941, Ray, Evans and Becks carried out a series of experiments on female rats. Hypophysectomy was done at varying ages from the active growth period to early adulthood. These animals were sacrificed at three age groups, 54, 88 and 150 days. In the 54 day old group, sacrificed 25 days post-hypophysectomy, the epiphyseal cartilage showed a decrease in width as compared with the normal controls. Histological changes were constant and marked in all the rats. In the zone of proliferating cartilage, the cells were reduced in size and number, and the nuclei were pyknotic. The columns of cartilage cells were regularly arranged but narrower than those in the unoperated controls of the same age. The zone of calcification was reduced in width, the cells appeared shrunken, and the lacunae were small. The zone of erosion was inactive, and some of the diaphyseal trabeculae had disappeared, the remaining trabeculae being large and well oriented. In the 88 day old group, also sacrificed 25 days post-hypophysectomy, the average decrease in width of the proximal tibial epiphyseal cartilage was even greater than in the 54 day old group (see Table 4). Histologically, regression in the epiphyseal disc and bone atrophy were more marked than in the younger animals. At 150 days of age, again following a post-hypophysectomy period of 25 days, the epiphyseal cartilage had become very irregular

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Table 4. The Effect of Hypophysectomy on the Proximal Tibial Epiphyseal Cartilage of Female Rats

	Age (days)	Unoperated Controls	Hypophysectomized
Width of Proximal Tibial Epiphyseal Cartilage	54	38	20
(One division equals 4.1 μ)	88	31	18
	150	40	27

(From Ray, Evans and Becks, 1941)

in width, and therefore measurements tended to lose their significance. Histologically, bone outlined both sides of the cartilage. In the zone of cartilage proliferation the size and number of cells were reduced and therefore the cartilaginous matrix was increased. The zone of calcification was narrow, the cells almost as small as those in the proliferative zone, and some even seemed to have become osteocytes. In the zone of erosion, bone had been deposited. The decrease in width of the epiphyseal cartilage in all three age groups of hypophysectomized female rats was proportional, therefore indicating that the result of ablation of the hypophysis was not passive cessation of growth but an active process resulting in premature ageing of the skeleton.

Evans, Simpson, Marx and Kibrick (1943) and Becks, Simpson, Li and Evans (1944) confirmed the previous findings in immature male rats. Becks, Simpson, Marx, Li and Evans (1944) obtained similar results on 26 to 28 day old hypophysectomized female rats.

In 1945, Becks, Simpson and Evans reported the changes in the proximal tibial epiphysis of female rats of the Long-Evans strain after post-hypophysectomy periods ranging from four to six hundred and seventeen days. Operation was done at 26 to 28 days of age. The results are best seen in Table 5. Decrease in cartilage width was progressive from four to 250 and 300 days after operation, but little further decrease in cartilage width occurred after this. Accompanying the decrease in cartilage width was a progressive decrease in the size of the cartilage cells and an increase in the amount of cartilaginous matrix with separation of the columns of cells. The cartilage continued to form an unbroken epiphyseal plate across the entire bone even at 617

Table 5. Width of Proximal Epiphyseal Cartilage of Tibia of Hypophysectomized Female Rats

No. Animals	Postop. Interval (days)	Age at Autopsy (days)	Cartilage Width (μ)
6	4	30	398
4	6	34	294
4	8	36	302
4	10	38	297
4	14	40	239
6	18	46	195
1	28	56	231
5	36	62	209
1	56	84	224
1	91	119*	198
8	100	126	215
1	132	160	178
4	145	168	208
1	167	195	186
24	205-210	230	211
1	272	300	173
40	300-330	341-392	160
14	442	470	156
2	617	650	153

* Measurements in older hypophysectomized rats include the calcified cartilage and true bone which seal the cartilage from the marrow.

(From Becks, Simpson and Evans, 1945)

days after operation. Early in the postoperative period, the bony trabeculae became coarser than those in the normal animal. As the post-operative interval increased, the trabeculae became more coarse and sparse, even more so than in normal old rats. These findings in the proximal tibial epiphysis at progressive post-hypophysectomy periods were comparable to those seen in ageing normal rats, with some marked differences. The rate of ageing in the epiphyseal region differed greatly. In the hypophysectomized animals atrophy and nearly complete disappearance of osteoblasts occurred almost immediately, in contrast to a much more gradual process in the normal animal. The width of the epiphyseal cartilage decreased quite abruptly at first and then rather slowly to about 272 days postoperatively, after which time it remained at a width greater than that in the normal rat of the same age. The bony trabeculae were more sparse than normal, and enclosed only small amounts of cartilage as compared with ample inclusions in the older normal rat. In the normal old rat some "sealing off" of the cartilage from the marrow occurred, but it was much more extensive and consistent in the hypophysectomized rat.

Ross and McLean (1940) administered an undifferentiated anterior pituitary substance to hypophysectomized rats of about six months of age, and found recognizable evidences of active growth in inactive or lapsed cartilage plates and adjacent spongiosa on histological examination.

That the anterior lobe of the pituitary gland contained a growth-promoting principle was established by Evans and his co-workers (1922, 1927), and since then their work has been

corroborated by Putnam, Teel and Benedict (1928), who used an alkaline extract of the anterior pituitary.

In 1939, Freud, Levie and Kroon extracted a highly purified substance from the anterior pituitary which was practically free of thyrotropic, corticotropic and prolactin activity, and which they felt to be a true growth-promoting fraction. Studies of the action on body weight in normal and hypophysectomized rats of a growth-promoting principle of the anterior pituitary had been made previously, and some work on skeletal growth had been done on guinea pigs by the Silberbergs (1935a, 1935b, 1936a, 1936b, 1936c, 1936d, 1937). However, Freud, Levie and Kroon (1939) chose to make observations on the effect of their fraction on tail growth in normal and hypophysectomized rats, and hypophysectomized rats treated with growth hormone, in an effort to localize the point of action of the growth hormone. They found that the growth defect following hypophysectomy was definitely localized in the epiphyseal cartilage of the tail of the growing rat, and that growth hormone administered immediately following hypophysectomy, and before the epiphyses had closed, resulted in a restoration of the normal condition. However, once the epiphyses had closed, treatment was unable to reverse the condition. These workers felt, therefore, that the point of attack of the growth hormone was localized in the proliferating cartilage, and that the terms growth hormone and chondrotrophic hormone were synonymous.

Continuing their series of experiments, Ray, Evans and Becks (1941) studied the effects of growth hormone on the proximal tibial epiphyseal cartilage of unoperated and hypophysectomized female rats

of three age groups, 54, 88 and 150 days at autopsy. In the unoperated animals at 54 days of age, there was a possible but not significant increase in cartilage width, and although the injected and control rats presented grossly similar findings, there did seem to be a definite increase in activity in the zone of erosion with reduction in the size of the trabeculae. In the operated animals at 54 days of age, however, there was a much greater response. The width of the epiphyseal cartilage was increased (see Table 6), and histological changes were even more marked than in the unoperated group. The cartilage cells were large, as were their lacunae, their nuclei karyolytic, and the cytoplasm vesicular. On the whole, they appeared less basophilic than usual. The columns of cartilage cells were longer than normal due to the fact that proliferation was greatly increased. Osteogenesis was extremely active, as well as chondrogenesis, as seen in the great increase in the number of cells in the zone of erosion and the numerous trabeculae covered with osteoblasts in the diaphyseal region. At 88 days of age, the unoperated group showed a very slight average increase in the width of the epiphyseal disc. Histological changes were much the same as in the 54 day old group, although the difference between injected and control animals was more marked than in the younger group. The width of the zone of proliferation was greater due to increase in the number and size of the cells, and the cytoplasm was less basophilic. The transition between the zone of proliferation and calcification was less abrupt, karyolysis of the nuclei proceeding gradually. As compared with their controls, the calcified zone of the injected animals was wider and the lacunae larger, even though the average number of cells remained the

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Table 6. The Effect of Growth Hormone on the Proximal Tibial Epiphyseal Cartilage of Hypophysectomized Female Rats

	Age (days)	Unoperated Controls	Unoperated, treated	Hypophysectomized, treated
Width of Proximal Tibial Epiphyseal Cartilage (One division equals 4.1μ)	54	38	45	73
	88	31	38	64
	150	40	55	56

(From Ray, Evans and Becks, 1941)

same. In the 88 day old hypophysectomized animals treated with growth hormone, the histological changes were exactly the same as those seen in the 54 day old group, but as compared with their controls the changes were more pronounced. The increase in over-all width of the epiphyseal cartilage was likewise more pronounced. At 150 days of age, the epiphyseal cartilage of the unoperated injected rats was markedly increased in width. Histologically the entire cartilage was hypertrophied and regular in width. There was evidence of rapidity of proliferation of cartilage cells, and in the zone of erosion activity was confined to a narrow band because in these older animals bone formation was relatively inactive prior to administration of hormone as compared with bone formation in the younger animals. In operated rats of 150 days, given growth hormone, the width of the cartilage was increased but not nearly so much as in the younger groups, and the histological changes were similar to those seen in the unoperated rats of the same age treated with the hormone, the age factor having its effect here also.

These experiments indicated, therefore, that administration of growth hormone to the normal animal with intact hypophysis had no marked effect on the proximal tibial epiphyseal cartilage, both cartilage and bone formation remaining in quite constant equilibrium. In the 150 day group, however, this equilibrium did appear to be upset somewhat as indicated by hypertrophy of the cartilage. This was due to the age factor, response of the cartilage predominating over activity in the zone of erosion. On the other hand, in hypophysectomized animals treated with growth hormone, the effects of pituitary deficiency were counteracted with a return to the "youthful" type of epiphyseal line

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as shown by the increased cartilage width and activity in the zone of erosion. Therefore, growth hormone reversed the regressive changes following hypophysectomy. The initial effect of treatment was increased cartilage formation with increase in the width of the disc during the first six to eight days, followed by re-establishment of the normal equilibrium between chondrogenesis and osteogenesis (Evans, Simpson, Marx and Kibrick, 1943).

In 1937, Moon found inhibition of somatic growth in young castrate male rats following administration of pituitary extracts rich in adrenocorticotrophic hormone (ACTH), and Ingle, Higgins and Kendall (1938) noted similar effects in normal rats treated with extracts of the adrenal cortex.

Marx, Simpson, Li and Evans (1943) found that administration of ACTH two weeks after hypophysectomy caused no increase in the width of the epiphyseal cartilage of the proximal end of the tibia of the rat. In fact, if anything, it caused a slight decrease in the width of the cartilage. These findings led to a more extensive study of the specific effect of ACTH on the epiphyseal cartilage (Becks, Simpson, Li and Evans, 1944).

Young male rats, 26 days of age, treated over a period of 30 days with pure ACTH showed a marked decrease in the width of the proximal tibial epiphyseal cartilage as compared with normal controls of the same age. Osteogenesis was likewise greatly retarded as compared with the normal animals who showed very active osteogenesis. The zone of erosion was irregular, and the large vacuolated cartilage cells were fewer in number. Likewise, the bony trabeculae of the diaphysis were irregular

in the treated animal, compared to the slender straight trabeculae in the normal rat. However, the decrease in width of the epiphyseal cartilage in treated normal animals was not so great as the decrease in width that followed hypophysectomy, and did not occur in the absence of the adrenals. Also, osteogenesis, although greatly retarded, continued at a reduced rate, whereas it ceased in hypophysectomized animals.

Since growth hormone was found to stimulate growth of the epiphyseal cartilage and ACTH to inhibit it, work was done to determine the results of simultaneous administration of these two hormones of the anterior pituitary. Marx, Simpson, Li and Evans (1943) found that administration of ACTH almost completely nullified the marked increase in width of the cartilaginous disc that occurred following administration of growth hormone, when both ACTH and growth hormone were administered simultaneously to female rats hypophysectomized at 26 to 28 days of age (see Table 7). Histological examination of the proximal tibial epiphyseal cartilage of young female rats hypophysectomized when 26 to 28 days of age and allowed a two-week postoperative period before treatment was started (Becks, Simpson, Marx, Li and Evans, 1944) revealed the following results when ACTH and growth hormone were administered simultaneously. The width of the cartilage was much less than that with growth hormone alone, and the vacuolated cells in the zone of calcification were somewhat larger than in the controls and showed evidences of extensive degeneration. In the zone of erosion the columns of cartilage cells were more irregular than normal. The bony trabeculae were crowded and irregular, and ended abruptly a short distance from the cartilage. Osteoblastic and osteoclastic activity were greatly decreased.

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Table 7. Effects of Pituitary Growth (GH) and
Adrenocorticotropic (ACTH) Hormones on the
Uncalcified Portion of the Proximal Epiphyseal Cartilage
of the Tibia in Hypophysectomized Rats

Daily Dosage Levels		Number of Rats	Width of Cartilage			
GH mg.	ACTH mg.		GH Alone (mm.)	GH + ACTH (mm.)	ACTH Alone (mm.)	Controls (mm.)
0.08	3.0	23	0.36	0.17	0.12	0.14
0.09	1.0	30	0.35	0.17	0.08	0.15
0.10	0.1	24	0.30	0.20	0.13	0.16

(From Marx, Simpson, Li and Evans, 1943)

The adrenocorticotrophic hormone is therefore antagonistic to the growth hormone in its effect on the epiphyseal cartilage, this antagonism serving to maintain a steady rate of growth in the normal state. It is felt (Becks, Simpson, Marx, Li and Evans, 1944) that due consideration must be given to the influence these two hormones exert in opposite directions on protein metabolism, growth hormone inducing a positive nitrogen balance (Teel and Cushing, 1930) and depression of liver arginase (Fraenkel-Conrat, Simpson and Evans, 1943) while ACTH causes a negative nitrogen balance (Long, Katzin and Fry, 1940) and activation of liver arginase (Fraenkel-Conrat, Simpson and Evans, 1943).

Interaction of Hormones in Relation to Bone Growth

Thyroid and Anterior Pituitary

Evans et al (1939) studied the interaction of the thyroid and pituitary glands in rats and found body growth (gigantism) resulting from administration of anterior pituitary extract to be more striking when the thyroid was present. They also found that anterior pituitary hormone when administered to thyroidectomized and thyroidectomized-hypophysectomized rats was much more effective when thyroid substance was added. Thyroxin did not stimulate growth when given to thyroidectomized-hypophysectomized animals. Since then, other results have been reported on the effect of anterior pituitary extract and thyroxin on the epiphyseal cartilage in thyroidectomized and thyroidectomized-hypophysectomized animals.

In 1942, Becks, Ray, Simpson and Evans thyroparathyroidectomized normal female rats at 35 to 45 days of age, following which they

were given daily doses of thyroxin, anterior pituitary extract (bovine) containing growth hormone, and a combination of both thyroxin and anterior pituitary extract. Thyroxin alone was followed by a return to the normal gross and histological picture from the condition resulting following thyroparathyroidectomy, with symmetric stimulation of chondrogenesis and osteogenesis. Anterior pituitary extract containing the growth hormone resulted in extremely active endochondral ossification. The balance between chondrogenesis and osteogenesis when established favored the former, with resultant increase in cartilage width. Administration of both thyroxin and anterior pituitary extract with growth hormone resulted in a more mature type of endochondral ossification with narrower epiphyseal cartilage and more rapid replacement of cartilage by bone. Synergism between the anterior pituitary extract containing growth hormone and thyroxin seemed evident, therefore, since the combination of hormones produced a more normal mature picture of endochondral ossification as compared with the picture seen in the animals treated with growth hormone and thyroxin alone. Another group of female rats were thyroparathyroidectomized and hypophysectomized at 35 to 128 days of age and given the same treatment. Thyroxin alone had no effect in returning the picture to normal, but the combined extracts did return the growth defect to normal. Anterior pituitary extract alone was not administered to this group.

In 1946, Becks, Simpson, Evans, Ray, Li and Asling hypophysectomized female rats of the Long-Evans strain at 26 to 30 days of age and maintained them for postoperative periods ranging from 271 to 344 days. Administration of growth hormone alone reawakened the

processes of bone growth with a marked increase in the proximal tibial epiphyseal cartilage and vigorous osteogenesis. Thyroxin alone had no appreciable effect on measurements of the epiphyseal cartilage. When both growth hormone and thyroxin were given simultaneously the cartilage increased slightly in width over that of the animals receiving growth hormone alone, but the difference was not significant. Bone formation was far more advanced than in any of the animals receiving growth hormone alone. However, it was recorded that both total body length and tibia length had increased significantly, and it was concluded that both proliferation of cartilage and erosion of calcified cartilage with bone replacement progressed at a rapid rate, setting up a new equilibrium which resulted in lengthening of the bone but no appreciable increase in the width of the cartilage alone, giving more evidence for the synergistic action of growth hormone and thyroxin.

Sex Hormones and Anterior Pituitary

The Silberbergs (1939), working with guinea pigs, have reported an apparent summation of the effects of castration and subsequent growth hormone therapy, with the difference that hyperplastic growth was accentuated in the male whereas hypertrophic growth predominated in the female.

Simpson, Marx, Becks and Evans (1944) hypophysectomized male rats at 40 to 41 days of age. In one group small doses of testosterone propionate were injected over a period of 20 days. The proximal epiphyseal cartilage of the tibiae were measured and were found to be as narrow in the uncalcified portion as those of the untreated controls. Histologically, capillaries had kept the cartilage columns open and some

new bone was being formed. Another group given growth hormone alone revealed the typical findings of wide, active epiphyseal cartilages with rapid erosion of calcified cartilage and deposition of bone. A third group was given both testosterone propionate and growth hormone, and the width of the proximal tibial epiphyseal cartilage was found to be essentially the same as that in the animals receiving growth hormone alone, although greater than that in the untreated controls. However, the tibiae were known to have increased more in length with the combined hormones than with growth hormone alone, and it seemed that an equilibrium had been reached in the 20-day period of injection (see Table 8). Female rats were also studied. Hypophysectomized at 26 to 29 days of age, testosterone propionate was injected for 10 days after a postoperative period of 42 to 90 days. The width of the proximal tibial epiphyseal cartilage was found to be significantly wider than in the controls, although the length of the entire tibia was the same in both. Microscopically, the zone of calcification was more distinct than in the untreated controls, with active capillary invasion and new bone formation. When testosterone propionate was administered for four days to females hypophysectomized at 26 days after a postoperative period of 13 days, no increase in the width of the uncalcified epiphyseal cartilage was seen, nor any difference histologically from the ossification process in the normal control. Growth hormone was administered alone and with testosterone propionate during the four-day period, and the epiphyseal cartilage was found in each group to have increased in width over the control to approximately the same degree (the same as in the males), with histological evidence of stimulation and vigorous bone formation. This

Table 8. Response of Tibia of Male Rats Hypophysectomized and Treated with Testosterone Propionate Alone and Combined with Growth Hormone*

Series I

Number of Rats	Treatment Substance	Treatment Daily dose (mg.)	Tibia Length (cm.)	Tibia Epiphyseal Cartilage ** (micra)
5	TP	0.25	3.30±0.03	112± 6.7
4	GH	0.12	3.38±0.03	280±13.0
5	TP + GH	0.25 0.12	3.50±0.05	251±19.9
4	Control	0	3.23±0.07	103± 8.6

Series II

Number of Rats	Treatment Substance	Treatment Daily dose (mg.)	Tibia Length (cm.)	Tibia Epiphyseal Cartilage (micra)
5	TP	0.25	3.33±0.05	121± 9.3
6	GH	0.25	3.49±0.05	267±31.2
6	TP + GH	0.25 0.25	3.61±0.06	279±17.1
5	Control	0	3.29±0.09	119± 9.21

*Operated at 40 to 41 days, treated for 20 days from time of operation

**Uncalcified portion of proximal epiphysis, silver nitrate stain.

(From Simpson, Marx, Becks and Evans, 1944)

was in contrast to the controls and the group receiving testosterone propionate alone (Table 9), where the epiphyseal cartilage and zone of erosion were quiescent. The authors claim that augmentation of the width of the proximal tibial epiphyseal cartilage, even though slight, in rats treated simultaneously with small doses of testosterone propionate and growth hormone indicates a synergistic action between the two hormones. From the data in Table 8, however, there does not appear to be augmentation of the cartilage width in treatment with testosterone propionate and growth hormone over cartilage width when treated with growth hormone alone. If anything, the opposite effect (inhibition or antagonism) appears to have resulted in Series I. However, in either series the differences in measurements between combined effects and effect of growth hormone alone do not appear to be very significant. Perhaps more definite evidence for the claim of synergistic action may be found in the increase in body weight and over-all body length noted in these animals.

In contrast to these results are those of Reiss, Fernandez and Golla (1946) who gave large doses of testosterone propionate alone and in combination with growth hormone to hypophysectomized rats (sex not mentioned) of the Wistar strain. Growth hormone alone (5 mg. daily dose) resulted in increased length of the tails of the rats with an increase of 23 per cent in body weight. Testosterone propionate alone (4 mg. daily dose) did not influence tail lengths nor the body weight decrease that usually occurs following hypophysectomy. However, when both were administered simultaneously, the rate of growth which resulted from growth hormone alone was reduced by more than 50 per cent, and body

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Table 9. Effect of Acute Injection (4 Days) of Testosterone Propionate Alone and Combined with Growth Hormone on the Width of the Epiphyseal Cartilage in Hypophysectomized 26 Day Old Female Rats

Series I

Number of Rats	Treatment Substance	Treatment Daily dose (mg.)	Body Weight Onset (gm.)	Body Weight Gain (gm.)	Epiphyseal Cartilage** (micra)
8	TP	1.00	68	2±0.55	169±11.6
8	TP	0.05	68	2±0.86	168±6.6
8	Control	0	67	1±0.50	169±5.0

Series II

Number of Rats	Treatment Substance	Treatment Daily dose (mg.)	Body Weight Onset (gm.)	Body Weight Gain (gm.)	Epiphyseal Cartilage (micra)
7	TP	0.25	68	0.4±0.68	165±4.1
7	GH	0.10	68	8±1.48	258±14.5
7	TP + GH	0.25 0.10	68	8±0.77	284±12.8
7	Control	0	68	1±0.81	163±8.95

** Uncalcified portion of proximal epiphysis, silver nitrate stain.

(From Simpson, Marx, Becks and Evans, 1944)

weight increase was considerably less. The proximal tibial epiphyseal cartilage was not measured. However, the usual pictures following hypophysectomy and administration of growth hormone were seen. In the animals given both growth hormone and testosterone propionate, there was no marked disturbance of the regular arrangement of the cartilage cells, but it was felt that the cells were perhaps slightly less active. The cartilaginous matrix was possibly comparatively more abundant than in the animals treated with growth hormone alone, and calcification and circulation were increased. The inhibitory effect of testosterone propionate on the action of growth hormone as shown by comparison of body weights and tail lengths does not appear to be very evident in the epiphyseal cartilage.

Kibrick, Simpson, Becks and Evans (1942) treated female rats hypophysectomized at 54 to 58 days of age with implants of pellets of alpha-estradiol dipropionate. They found no histological differences between the tibiae of the hypophysectomized rats treated with estrin and those that were not treated. The typical findings under the normal influence of estrin, namely an increase in the number and size of bony trabeculae making up the primary spongiosa, were not seen. Rather, the typical findings of resorption of primary spongiosa and retention of secondary spongiosa which follow hypophysectomy were seen in both the treated and untreated animals. Nothing resembling the effects of estrin on the histology of the proximal tibial epiphyseal cartilage previously described could be demonstrated in the absence of the pituitary.

When Reiss, Fernandex and Golla (1946) gave 0.1 mg. of oestrone daily in combination with 5 mg. of growth hormone daily to hypophysectomized

weight increase was considerably less. The proximal tibial epiphyseal cartilage was not measured. However, the usual pictures following hypophysectomy and administration of growth hormone were seen. In the animals given both growth hormone and testosterone propionate, there was no marked disturbance of the regular arrangement of the cartilage cells, but it was felt that the cells were somewhat slightly less active. The cartilaginous matrix was possibly comparatively more abundant than in the animals treated with growth hormone alone, and calcification and circulation were increased. The inhibitory effect of testosterone propionate on the action of growth hormone as shown by comparison of body weights and tail lengths does not appear to be very evident in the epiphyseal cartilage.

Kibrick, Simpson, Becks and Evans (1942) treated female rats hypophysectomized at 24 to 30 days of age with implants of pellets of alpha-estradiol dipropionate. They found no histological differences between the tibiae of the hypophysectomized rats treated with estrin and those that were not treated. The typical findings under the normal influence of estrin, namely an increase in the number and size of bony trabeculae making up the primary spongiosa, were not seen. Rather, the typical findings of resorption of primary spongiosa and retention of secondary spongiosa which follow hypophysectomy were seen in both the treated and untreated animals. Nothing resembling the effect of estrin on the histology of the proximal tibial epiphyseal cartilage previously described could be demonstrated in the absence of the pituitary.

When Reiss, Fernandes and Golla (1946) gave 0.1 mg. of oestrone daily in combination with 5 mg. of growth hormone daily to hypophysectomized

rats of the Wistar strain, they obtained similar results to those when testosterone propionate and growth hormone were given simultaneously. That is, tail length growth and body weight were inhibited. However, changes in the proximal tibial epiphyseal cartilage were not recorded.

Adrenal Cortical Hormones and Anterior Pituitary

Except for those experiments on the interaction of the adrenocorticotrophic and growth hormones discussed previously, the only reports on the interaction of the anterior pituitary and adrenal cortical hormones is that of Reiss, Fernandez and Golla (1946), who gave desoxycorticosterone acetate (DOCA) and growth hormone simultaneously to hypophysectomized rats of the Wistar strain. They found inhibition of the effects of the growth hormone by DOCA as shown by loss of body weight and decreased tail length. This inhibition was not as great, however, as in the case of simultaneous administration of testosterone propionate and growth hormone. Unfortunately, effects on the proximal tibial epiphyseal cartilage were not described.

Information on the interaction of other hormones, such as the adrenocortical and sex hormones, adrenocortical and thyroid hormones, and thyroid and sex hormones, in relation to bone growth and particularly cartilage has not been reported to my knowledge. Plans are now being made at the Boston University Biology Research Laboratory to investigate the relationship between the adrenocortical and sex hormones.

Baker and Leck (1946) have ruled out any interaction between the parathyroid and sex hormones by showing that injection of estrogens

in parathyroidectomized rats produced changes in the epiphyseal cartilage and subepiphyseal region which were of similar magnitude and character to those produced in normal estrogen-treated rats.

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PROPOSED EXPERIMENTAL APPROACH

In February, 1948, under the direction of Dr. Leland C. Wyman, an investigation of the effects of bilateral adrenalectomy on the proximal tibial epiphyseal cartilage of male rats of the Wistar (albino) strain was started at the Boston University Biology Research Laboratory. Previously (1945), Dr. Wyman and Dr. Caroline tum-Suden had studied this problem in rats of the Long-Evans strain at the Boston University School of Medicine, Department of Physiology. The original research program this year was planned to prepare microscopic sections of the proximal end of the tibia for histological study of the effects of adrenalectomy on the epiphyseal cartilage of Long-Evans rats, since only ocular micrometer measurements of the cartilage of silver impregnated bones have been made to date. However, we experienced difficulty in obtaining these animals from the usual source on short notice, and we turned our attention to rats of the Wistar strain which could be very easily and speedily procured in Boston. The present investigation was directed primarily, therefore, to a comparison of the effects of adrenalectomy in rats of the Wistar and Long-Evans strains, with the idea of replacing the Long-Evans rats with the Wistar strain in future experimental work if the comparison proved favorable. We would also have an opportunity to compare strain differences, if they occur, and would be provided with material for microscopic study of the proximal end of the tibia.

Male rats were obtained from the breeding laboratories shortly after they had been weaned, and were maintained in our laboratory on

Rockland Rat Diet. Approximately two-thirds of the animals were adrenalectomized, and one-half of these placed on saline drinking water while the other half were given tap drinking water. The other one-third of the animals were blank operated and kept as controls.

Adrenalectomy was performed at ages varying from 29 to 39 days, under ether anesthesia, through a dorsal midline skin incision. With sharp-pointed forceps, the muscles on either side of the vertebral column were split in the direction of their fibers over the kidney region, just distal to the last rib. The kidney was exposed, and the adrenal located in the perirenal fat and removed, care being taken not to leave any adrenal tissue. If the capsule was thought to be ruptured, or any adrenal tissue whatsoever thought to have been left, the animal was kept as a blank operated control. The blank operated control animals were anesthetized with ether and exactly the same procedure carried out as in the experimental animals, except that the adrenals were not removed. Thus any changes due to operative procedure alone could be ruled out. A few animals were kept as unoperated normal controls.

As soon as the animals died or were sacrificed, the left tibia was removed and split sagittally. Whenever possible, the bones were prepared for silver staining immediately, but in most instances the sections were placed in five percent formalin until conditions were suitable for staining. The silver impregnation technique used was that recommended by Evans, Simpson, Marx and Kibrick (1943) and used by Wyman and tum-Suden (1945) in their series. The bones were first washed in water, then placed in acetone and washed again. Following this, the sections were immersed in two percent silver nitrate solution, in direct

sunlight, for a very short time. During the staining process, calcium phosphate in the calcified areas is replaced by silver phosphate, which is then reduced to black metallic silver by washing the sections in sodium thiosulfate solution. The bones were then washed again following the staining procedure. The appearance of the proximal end of the tibia is that seen in Fig. 1, the uncalcified areas remaining clear and colorless, the calcified areas standing out as black or brownish in color. Measurement of the uncalcified epiphyseal cartilage was made by means of an ocular micrometer, six readings being taken, one at either end and the others at regular intervals across the cartilage, and then averaged. The right tibia was removed and placed in Zenker's fixative (containing acetic acid), to be decalcified and stained later with hematoxylin and eosin.

The adrenalectomized animals on tap drinking water succumbed to adrenal insufficiency from one to fifteen days after operation. The proximal tibial epiphyseal cartilage of the one animal (No. 16) that died one day post-adrenalectomy was much narrower than that of four normal controls of the same age, and death may be considered due to factors other than adrenal insufficiency, possibly inanition due to change of diet since the diet at the breeding laboratories was not the same as that fed to the rats in our laboratory. Of the adrenalectomized animals on saline drinking water, one (No. 4) died some time over a week-end, two to four days postoperatively, and the silver stain of the epiphyseal cartilage was very poor. Another (No. 33) died three days post-adrenalectomy with signs of digestive disturbance. Evidence of digestive disturbance was seen also in animal No. 13, which died twelve days

postoperatively. In view of the short postoperative periods and the possibility of other factors obscuring the picture, these animals are omitted from the tabulated results. Two of the unoperated normal controls (Nos. 21 and 42) had definitely narrower cartilages and appeared smaller than others of the same age, and are therefore not included in the results. In three other animals (Nos. 1, 2 and 6), the silver stain of the bones was so poor that readings were impossible.

As may be seen from Tables 10 and 11, and Fig. 12, the width of the proximal tibial epiphyseal cartilage of adrenalectomized rats on tap drinking water is noticeably narrower than that of their blank operated and normal controls. However, the difference is not as great as that found by Wyman and tum-Suden (1945) in the Long-Evans strain. The width of the cartilage in adrenalectomized animals on saline drinking water is only slightly increased over that of the control animals, and in some cases appears to be even less. However, the general trend is that of increased cartilage width, but to a lesser degree than that found in the Long-Evans rats. Therefore, the curves seen in Fig. 12 may be interpreted as indicating a tendency to increased cartilage width in adrenalectomized rats of the Wistar strain on saline drinking water, as compared with control animals. Whether or not this tendency will prove to be the actual case remains to be seen. Too few animals have been studied to draw any definite conclusions, and the possibility of other factors entering into the picture cannot be overlooked. Although the animals were of known age and sex, litter-mates were not segregated, and change of diet, even at so short a period after weaning, may have had some effect.

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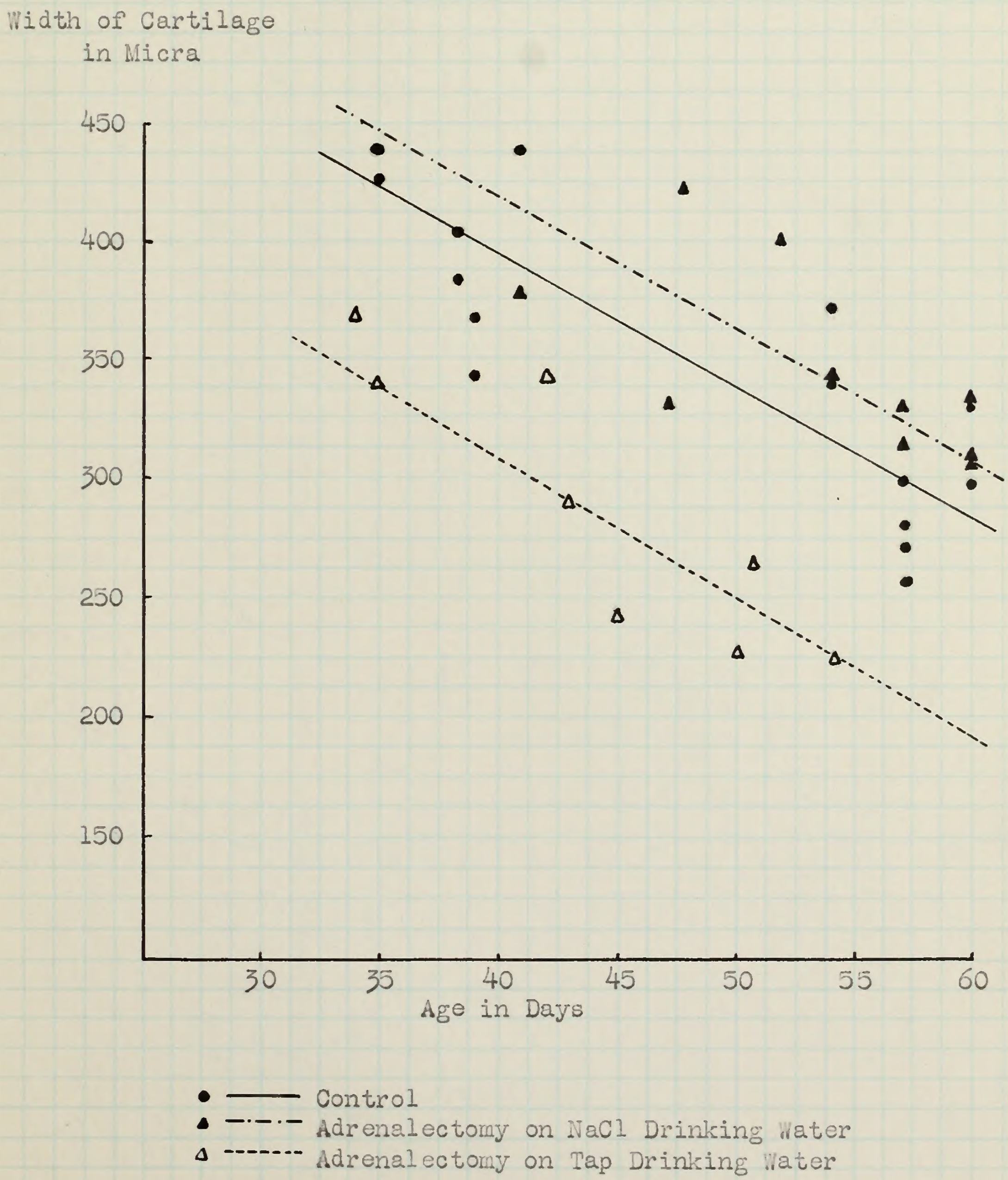
Table 10. Effect of Adrenalectomy on the Proximal Tibial Epiphyseal Cartilage of Rats of the Wistar Strain

Experimental Procedure	No. of Rats	Age at End of Exper. (days)	Postop. Period (days)	Width of Cartilage (micra) Range	Average
Normal Controls	2	38		385-405	395
	2	39		344-368	356
Blank Operated Controls	2	35	5	425-440	433
	1	41	12		440
	2	54	16	338-371	355
	4	57	18	256-299	276
	2	60	17	298-332	315
Adrenalectomy on NaCl Drinking Water	1	41	12		378
	1	47	8		332
	1	48	9		423
	1	52	13		401
	1	54	16		343
	2	57	18	315-329	322
	3	60	17	312-336	314
Adrenalectomy on Tap Drinking Water	1	34	4		364
	1	35	5		340
	1	42	3		343
	1	43	4		289
	1	45	6		244
	1	50	11		234
	1	51	12		266
	1	54	15		232

Table 11. Effect of Adrenalectomy on the Proximal Tibial Epiphyseal Cartilage of Rats of the Wistar Strain

Experimental Procedure	No. of Rats	Age at End of Exper. (days)	Postop. Period (days)	Width of Cartilage (micra) Range	Average
Controls	6	35-39		344-440	395
	8	54-60		256-371	305
Adrenalectomy on NaCl Drinking Water	3	41-48	8-12	332-423	378
	7	52-60	13-18	312-401	336
Adrenalectomy on Tap Drinking Water	2	34-35	4- 5	340-364	352
	3	42-45	3- 6	244-343	292
	3	50-54	11-15	232-266	244

Fig. 12. The Effect of Adrenalectomy on the Proximal Tibial Epiphyseal Cartilage of the Rat (Wistar Strain)



However, the difference between the results of this experiment and that of Wyman and tum-Suden (1945) may be due to variation in my technique as compared with theirs. Therefore, the same procedure is being repeated on a few Long-Evans rats to evaluate the findings on the Wistar rats. If measurement of the cartilage in the present series of Long-Evans rats is compatible with the measurements obtained by Wyman and tum-Suden, with greater increase and decrease in cartilage widths than that obtained in the Wistar rats, a strain difference in reaction to adrenalectomy will be evident. On the other hand, if results obtained in the Long-Evans rats correspond to those obtained in the Wistar rats, the difference between my results and those of Wyman and tum-Suden will necessarily be shown to be due to variation in my technique, and not to strain differences.

The decreased cartilage width in adrenalectomized animals on tap water was demonstrated by Wyman and tum-Suden (1945) to be due to inanition as the result of loss of appetite and decreased food consumption, in addition to adrenocortical insufficiency. In salt treated animals, however, appetite and food consumption are maintained, and Wyman and tum-Suden suggested that the increased cartilage width might be explained on the basis of the "end-organ" hormone hypothesis. This may be defined as a reciprocal or antagonistic effect between the "end-organ" in question and the anterior pituitary gland. Production of an excessive amount of hormone of the "end-organ", or gland which is stimulated, has a suppressing effect on production of the anterior pituitary trophic hormone which stimulates the "end-organ". Not only is this effect directed to the specific trophic hormone, but to anterior

pituitary activity in general. Thus, in the case of the adrenal, increased production of adrenocorticotropic (ACTH) and growth hormones by the anterior pituitary would be suppressed by increased production of adrenocortical hormone. By extending this hypothesis, removal of any "end-organ" which is stimulated by an anterior pituitary trophic hormone might be expected to "release" all the trophic hormones of the anterior pituitary as well as the one for the "end-organ" involved. Thus, removal of the adrenals would result in release, not only of its own trophic hormone, but of growth hormone as well, causing increase in the width of the cartilage. We might also expect the same results when the gonads are removed; that is, release of the growth hormone, with a summation effect if gonads and adrenals are removed simultaneously (Fig. 13). With this in mind, we are now making plans to study the relationship between gonads, adrenals and anterior pituitary gland to the growth of bone. This will be undertaken as soon as the present work on comparison of the effect of adrenalectomy on the Wistar and Long-Evans strains is completed.

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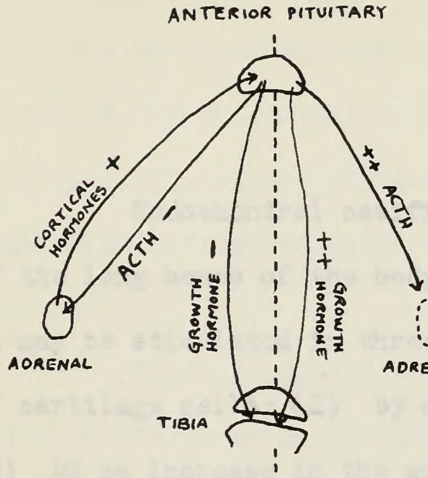
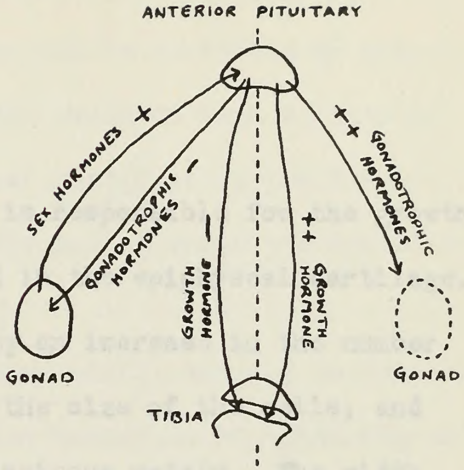


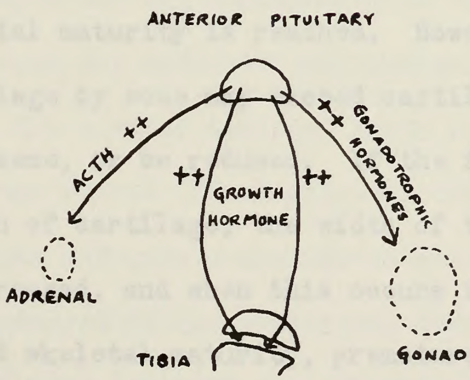
Excess amounts of cortical hormone suppress production of trophic hormones by the anterior pituitary.

Removal of adrenals releases growth hormone as well as ACTH.



Excess amounts of sex hormones suppress production of trophic hormones by the anterior pituitary.

Removal of the gonads releases the growth hormone as well as gonadotrophic hormones.



Removal of both adrenals and gonads simultaneously should give summation effect.

Fig. 13. Interpretation of expected results of adrenalectomy and gonadectomy on the proximal tibial epiphyseal cartilage of the rat, on the basis of the "end-organ" hormone hypothesis.

DISCUSSION

Endochondral ossification, which is responsible for the growth of the long bones of the body, is initiated in the epiphyseal cartilage. It may be stimulated in three ways: (1) by an increase in the number of cartilage cells; (2) by an increase in the size of the cells; and (3) by an increase in the amount of cartilaginous matrix. The width of the cartilage may be affected in two ways: (1) by variation in the cartilage itself by one or more of the three types of stimulation previously mentioned, or (2) by replacement of cartilage by bone. In the normal state, cartilage and bone formation remain more or less in equilibrium during the growth period, with bone formation becoming predominant as skeletal maturity is reached. However, the subsequent replacement of cartilage by bone may exceed cartilage formation, remain proportionately the same, or be reduced. If the formation of bone exceeds the formation of cartilage, the width of the epiphyseal cartilaginous disc is decreased, and when this occurs in the young animal before it has reached skeletal maturity, premature ageing of the skeleton with stunting results. If the two processes remain in equilibrium, the width of the disc remains within normal limits but the amount of cancellous bone increases as the animal matures. If the formation of cartilage exceeds the formation of bone, the width of the epiphyseal disc increases, and the proximal end of the tibia, for example, retains a more youthful appearance than is normal for the age of the animal, resulting in lengthening of the bone and therefore of

the skeleton in general. One other condition may occur; that is, formation of cartilage and formation of bone may be inhibited or prevented to the same degree. The growth of the skeleton then remains at a standstill, and the width of the epiphyseal cartilage varies from that of the normal animal, depending upon the age at which the inhibition occurs.

These irregularities are easily discernible and can be compared quite favorably using the silver impregnation technique of measuring the uncalcified cartilage with the ocular micrometer. Such measurements were made in experiments on the action of several of the hormones on growth of the cartilage, and compare favorably with the findings on histological examination. However, the condition may arise where cartilage and bone formation exceed the normal rate but remain in equilibrium, so that the bone increases in length but the measurement of the uncalcified cartilage by silver technique does not indicate the increased rate of growth. This was the case in the work of Simpson, Marx, Becks and Evans (1944) where they compared the effect of simultaneous administration of testosterone propionate and growth hormone in male rats. According to the micrometer measurements of the epiphyseal cartilage, in one series there was evidence of some inhibition of the growth hormone by testosterone propionate. However, measurement of over-all body length and length of the tibia indicated that the animals were growing at a faster rate under the influence of the combined hormones than with growth hormone alone, leading to the conclusion that these hormones were synergistic in their action on bone growth. In the second series, measurement of tibia length and epiphyseal cartilage width indicated a

synergistic action of the two hormones, but histological examination of the epiphyseal region revealed no augmentation of the effects of growth hormone by the addition of testosterone propionate.

Becks, Simpson, Evans, Ray, Li and Asling (1946) found similar results in their work with growth hormone and thyroxin. When both hormones were given together the increase in cartilage width over that in the animals receiving growth hormone alone was not significant, and one would be tempted to draw the conclusion that thyroxin had no effect in the presence of growth hormone. However, when the length of the tibia and of the body as a whole was considered, it was seen that growth was greatly augmented and the hormones were synergistic in their action on bone growth, setting up a new equilibrium between cartilage and bone formation.

Therefore, measurement of the epiphyseal cartilage alone may not always be an accurate indication of the rate of bone growth, and should be reinforced by histological examination to locate the site of experimentally or pathologically produced disturbance. Even these two procedures may not give a true picture of what is taking place if chondrogenesis and osteogenesis remain in equilibrium but progress at a faster rate than normal. Changes in body weight and over-all body length, together with measurement of the tibia, for instance, if that is the bone under consideration in the experiment, should be correlated with histological examination and micrometer measurement of the cartilage. On the other hand, comparison of body length and tibia length alone without histological examination will not determine the point of altered activity in the growth of the bone.

A problem which complicates the interpretation of the specific action of some of the hormones on bone growth is that of maintenance of an adequate diet and normal food consumption. Saxton and Silberberg (1947) have shown quite conclusively that enrichment of the diet beyond that adequate for normal growth results in acceleration of growth and premature ageing of the cartilage. Also, inadequate food consumption causes narrowing of the epiphyseal cartilage; however, growth is not prematurely arrested but slowed in rate. Difficulty with the problem of reduced food consumption was encountered by Wyman and tum-Suden (1945) in adrenalectomized rats, but it was overcome by the administration of saline drinking water which returned the appetite and food consumption of the rats to normal. Thus, their findings of increased cartilage widths in adrenalectomized rats whose food intake was adequate was the reverse of the results of Ingalls and Hayes (1941), who found decreased cartilage widths but failed to rule out the effects of inanition on cartilage growth.

It is fortunate that in the case of adrenalectomy salt treatment returns the appetite and food consumption to normal. In the case of administration of estrogen, however, the problem is not so easily solved. Simpson, Kibrick, Becks and Evans (1941) found conditions in the epiphyseal cartilage following estrogen treatment resembling those following starvation, and noted that food consumption appeared to be less than normal. The Silberbergs (1941) obtained similar results and noted failure of the animals to gain weight, but food consumption was not measured. Day and Follis (1941) demonstrated that changes occurred in the epiphyseal cartilage following restricted food intake which were

similar to, although less marked than changes following intensive estrogen treatment. Baker and Leck (1946), also, have concluded that inhibition of epiphyseal cartilage growth may be the result of partial inanition resulting from the toxicity of high doses of hormone, and thus the true effect of the hormone on bone growth is masked.

The age at which the animal is deprived of its endocrine organs or treated with hormones determines to a large extent the changes which occur in bone growth. The younger the animal, as a rule, the more severe are the effects. Up to the time when the animal has reached its full growth, both chondrogenesis and osteogenesis may be stimulated or depressed. However, once the animal is fully grown, osteogenesis is not greatly effected, but since the epiphyseal cartilage persists well into old age in the rat, changes in the cartilage may be produced, although not to as great an extent as in the younger animal.

The fundamental endocrinological principle which establishes the anterior pituitary gland as the master gland of all bodily functions is well illustrated in the study of the relationship of the various hormones to the growth of bone. Hypophysectomy has been conclusively shown to result in practically complete cessation of growth (Ray, Evans and Becks, 1941; Evans, Simpson, Marx and Kibrick, 1943; Becks, Simpson, Li and Evans, 1944; Becks, Simpson and Evans, 1945), even when all other endocrine organs are present, and administration of hormones other than those of the anterior pituitary can in no way compensate fully for the loss of anterior pituitary hormones. That there is a specific growth-promoting principle produced by the anterior pituitary gland has like-wise been shown (Evans and co-workers, 1922, 1927; Freud, Levie and

Kroon, 1939; Ray, Evans and Becks, 1941), with the isolation of the growth hormone which has been demonstrated to be chondrotrophic in action.

The reaction of the proximal tibial epiphyseal cartilage of the rat to disturbance in endocrine balance may be explained, in some instances at least, on the basis of the "end-organ" hormone hypothesis, described previously. As already stated, Wyman and tum-Suden (1945) felt that the increased cartilage width found in saline-treated adrenalectomized animals was due to release of growth hormone from the anterior pituitary. Activity in the opposite direction, that is depression of chondrotrophic activity, may possibly be an alternative explanation of the inhibitory effect produced by administration of ACTH to normal and hypophysectomized animals (Becks, Simpson, Li and Evans, 1944; Marx, Simpson, Li and Evans, 1943; Becks, Simpson, Marx, Li and Evans, 1944). The inhibition of growth was much more marked in the hypophysectomized animals as compared with the normal animals treated in this manner. This is as might be expected, since production of growth hormone and ACTH are entirely stopped, but when ACTH is administered to the normal animal it doubtless stimulates the adrenal cortex to produce its hormone in excessive amounts and thus may indirectly inhibit anterior pituitary activity and growth hormone production.

Retarded endochondral bone formation, and diminished proliferation and degeneration of cartilage cells and removal of calcified cartilage in normal animals given excessive amounts of thyroid hormone (Smith and McLean, 1938), may be explained on the basis of this hypothesis, also. That is, excessive thyroid hormone suppresses trophic

activity of the anterior pituitary and growth production is inhibited, resulting in premature cessation of growth. In the case of absence of the thyroid gland, however, evidence supporting the "end-organ" hormone hypothesis is lacking. Micrometer measurements were not made on the epiphyseal cartilage, but on the basis of histological reports and the gross observations of stunting in size of thyroidectomized animals (Laqueur, Dingemanse and Freud, 1941; Salmon, 1941; Becks, Kibrick and Evans, 1942), chondrotrophic activity of the anterior pituitary appears to be suppressed. Maturation of cartilage seems to be held at a standstill, while endochondral bone formation is also slowed (Becks, Kibrick and Evans, 1942). Rather, the results following thyroidectomy indicate a direct action of the thyroid hormone on the growth of bone. If, according to the "end-organ" hormone hypothesis, trophic hormones are released from the anterior pituitary, including growth hormone, the effect must be greatly overshadowed by the disturbance of body metabolism as the result of thyroid deficiency. Direct action of the thyroid gland on bone growth may be further substantiated by the demonstration of synergism between the thyroid and growth hormones (Becks, Ray, Simpson and Evans, 1942; Becks, Simpson, Ray, Li and Asling, 1946).

The effect of gonadectomy, namely stimulation of hyperplasia and hypertrophy of cartilage cells and calcification of hypertrophic cartilage, is explained by the Silberbergs (1939) on the basis of the "end-organ" hormone hypothesis. That is, removal of the gonads results in an increased production or liberation of growth hormone, thus stimulating bone growth. The extent to which production of the growth hormone is stimulated apparently depends on the sex of the animal, since

cartilage proliferation was more intense in the males than in the females, indicating exercise of stronger inhibition of the testicle on the anterior pituitary than of the ovary. Administration of estrogens to young male rats and to female rats with intact ovaries (Simpson, Kibrick, Becks and Evans, 1941; Silberberg and Silberberg, 1941a; Day and Follis, 1941) may cause a suppression of the anterior pituitary trophic hormones, resulting in shrinkage of the proximal tibial epiphyseal cartilage, although the picture is complicated by inadequate food consumption. Administration of testosterone propionate in excess (Silberberg and Silberberg, 1941b) apparently depresses chondrotrophic activity of the anterior pituitary, resulting in inhibition of proliferation and growth of cartilage cells and increased degeneration of cartilage cells, and increased sclerosis, hyalinization and calcification of the cartilaginous matrix. The difference in results obtained with smaller doses of testosterone propionate (Rubinstein, 1940; Rubinstein and Solomon, 1941b) and with large doses (Rubinstein, Kurland and Goodwin, 1939; Rubinstein and Solomon, 1941a) may be explained by a difference in the mode of action of the androgen on the anterior pituitary dependent on concentration of the gonadal hormone. That is, a small amount of the male sex hormone may stimulate anterior pituitary activity, while large amounts suppress it, or what is more likely, small amounts of testosterone propionate may not effect anterior pituitary activity but may have a direct effect on growth. Bearing out the latter assumption is the apparent finding of synergism between testosterone propionate and growth hormone and the absence of synergism between estrogen and growth hormone in the experiments of Simpson, Marx, Becks and Evans (1944) and Kibrick, Simpson,

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Becks and Evans (1942), from which it appears that testosterone propionate has a direct effect on bone growth while the action of estrogen is mediated through the anterior pituitary with no direct effect on growth. However, the results of the work on the relation of the sex hormones to bone growth are inconclusive, and the problem requires expansion and clarification.

Whether or not the effects of the adrenocortical hormones are mediated only through the anterior pituitary, or whether they may have a direct effect on bone growth, is open to question. As stated above, it has been shown that the adrenocorticotropic hormone of the anterior pituitary is antagonistic in its action on growth stimulated by the growth hormone (Marx, Simpson, Li and Evans, 1943; Becks, Simpson, Marx, Li and Evans, 1944). However, simultaneous administration of desoxycorticosterone acetate and growth hormone in the absence of the pituitary (Reiss, Fernandez and Golla, 1946), results in inhibition of the effects of growth hormone on body weight and tail length, and the question of direct action on growth may be pondered. The effects of the action on the epiphyseal cartilage and growth of bone of growth and adrenocortical hormones in the absence of the pituitary remains a problem for future investigation.

Since it is a generally accepted, although still controversial, theory that the parathyroid and anterior pituitary glands function independently, study of their combined effects on bone growth is not especially indicated at this time.

In any problem involving the function of the endocrine glands, due consideration must be given to the fact that disturbance in the

function of one gland upsets the function of the entire endocrine system. Thus here, a true evaluation of the effects of the various hormones, produced experimentally, must include a consideration not only of the effects of the hormones alone on the growth of bone, but also the effects of such factors as inanition, and disturbance of metabolism and of bodily functions as a whole, a discussion of which extends beyond the scope of this paper.

ABSTRACT

This paper considers the effects of the secretions of the endocrine glands on bone growth, with particular attention directed to the proximal tibial epiphyseal cartilage.

A brief review of the events leading to the present-day knowledge of the influence of the hormones on bone growth has been given. The material discussed has been limited mainly to the results of work on rats.

As a basis for later comparison of abnormal with the normal conditions, a detailed account of the embryonic and later development of bone has been included. Five differentiated areas in the proximal tibial epiphyseal cartilage, important in the localization of the points of experimentally caused disturbances, are noted.

Changes occur in the epiphyseal disc as the animal grows to maturity. In the young animal the disc is quite wide, but becomes increasingly irregular, is seen to decrease abruptly in width at three and a half to four months of age, and then to decrease more slowly as adulthood is reached. In the rat, the epiphyseal cartilage persists well into old age, even though the epiphysis becomes sealed off from the diaphysis.

Strain differences in the rat have not been noted in the study of internal secretions and bone growth, and definite conclusions cannot be reached at this time on the basis of the literature reported here.

Sex differences in the normal processes of growth are not obvious in reports by many workers. There is positive evidence in only one experiment which was conducted with a view to determination of differences between the sexes. Skeletal development was found to progress at a faster rate in females of the Osborne Mendel (Yale) strain of rats than in males. It is suggested that similar study of the skeletal development of both sexes of other strains may reveal similar results.

That diet and adequate food intake are important in maintaining the normal rate of growth is shown by the changes in activity in the epiphyseal cartilage which occur following enriched diets and adequate and inadequate food intake. Growth and ageing of cartilage may be accelerated by enriched diets, or prolonged by inadequate food consumption.

The result of bilateral adrenalectomy was first seen to be a decrease in cartilage width caused by inhibition of cartilage proliferation and maturation, and inhibition in the formation of bone. However, when food intake was maintained at a normal level by salt treatment in the form of saline drinking water, the width of the epiphyseal cartilage was seen to become increased as compared with normal controls. Histological sections of the proximal tibial epiphyseal cartilage of bilaterally adrenalectomized rats are now being prepared.

The effect of thyroparathyroidectomy in young rats is a retardation of maturation of epiphyseal cartilage and slowing up of endochondral bone formation, with resultant dwarfing. Administration of thyroid repairs the dwarfing caused by thyroidectomy by symmetric

stimulation of chondrogenesis and osteogenesis. Toxic doses of thyroid given to animals with intact thyroid glands causes premature cessation or retardation of endochondral bone formation.

The problem of the relation of parathyroids to bone growth is concerned for the most part with calcium metabolism. Some comparisons have been made between the effects of thyroparathyroidectomy and parathyroidectomy, with conflicting results. Further study is needed to clarify the issue, with more strict attention to the effects of thyroid and parathyroid deficiencies and treatment separately and in combination.

The results of work with the sex hormones likewise are confusing, and further work is needed. However, gonadectomy appears to maintain a youthful epiphyseal cartilage in guinea pigs. Specific effects of gonadectomy on the epiphyseal cartilage of rats have not been reported, to my knowledge. Treatment with estrogen causes an involution of the proximal tibial epiphyseal cartilage in young female rats immediately following treatment, with recovery of growth capacity and hyperossification as time goes on, the degree of hyperossification depending on the length of treatment. Still later the excessive bone is absorbed, and the end result is premature ageing of cartilage and bone formation with stunting in size. The results of administration of testosterone propionate apparently depend upon the dosage. Large amounts of the hormone result in a depressing or inhibitory influence on body growth, whereas small amounts appear to stimulate growth. In mice, proliferation and growth of cartilage, and bone formation and absorption are inhibited. Work on rats is not reported, as far as I

have been able to determine.

Hypophysectomy causes regression of the epiphyseal disc and bone atrophy, with resultant premature ageing of the skeleton. Administration of growth hormone causes a return to normal in the epiphyseal cartilage of the hypophysectomized animal, but has no marked effect in the animal with intact hypophysis.

Antagonism between the adrenocorticotropic and growth hormones of the anterior pituitary on the epiphyseal cartilage has been described. A synergistic action between the thyroid and growth hormones has been reported. What appears to be a synergistic action between testosterone propionate and growth hormone is considered. Estrogen appears to have no effect on growth when given to hypophysectomized animals. Desoxycorticosterone acetate is seen to inhibit the effects of growth hormone on body weight and tail length in hypophysectomized animals, but the effect on the proximal tibial epiphyseal cartilage is not reported.

Factors to be considered in interpreting the results of experiments on the relation of internal secretions to bone growth are discussed.

The endocrinological principle which establishes the anterior pituitary as the master gland of the body is well illustrated in the effects of the hormones on bone growth. Interpretation of the reaction of the proximal tibial epiphyseal cartilage to disturbance in endocrine balance on the basis of the "end-organ" hormone hypothesis and of direct action on cartilage is considered.

Suggestions for future investigation are made.

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